Int. J. Dev. Biol. 42: 531-533 (1998)

Serendipity, the principle of limited sloppiness, and neural development

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Our first encounter with Antonio García-Bellido was around 1974, when we had just begun our postdoctoral studies in Seymour Benzer's lab. Antonio was spending some time then as a visiting Professor at Ed Lewis' Lab in Caltech. Even for novices like ourselves, we were aware that Antonio had already done important things in Developmental Biology. Just a year earlier, he had published the classic paper on compartments (García-Bellido *et al.,* 1973). As our intention was to study fly neurobiology in Seymour's laboratory, we did not think that Antonio's world and our world had much in common. We certainly had no inklings that Antonio would have a strong influence on our direction many years later.

In 1974, fly neurogenetics was far from the mainstream of neurobiology. Perhaps because of that, people who were attracted to Seymour's lab were an eclectic and fairly creative group. We were fortunate to be included in Seymour's group with three other postdocs: Alain Ghysen, Ilan Deak and Yadin Dudai, as well as three graduate students: Don Ready, Bill Harris and Ducan Byer. One small act of Seymour's that turned out to influence us profoundly was his assignment of our benches right next to Alain's bench. One day after a seminar, we were mouthing off our opinions about the seminar with Bill Harris. Alain, who was sitting at the next bench quietly, suddenly spoke up and stunned us by shredding our arguments. From that moment on, we learned to think a little before opening our mouths in his presence. If it were not for the proximity of our benches, we might not have gotten to know Alain so well and to appreciate his sharp intellect, because we and Alain were all relatively quiet in the setting of larger groups.

One day during that year, Antonio decided to give a talk about the subject that was his main interest during his stay at Caltech. We all went to hear him. It was a fascinating and at the same time demoralizing experience for us. Antonio talked about the *achaete-scute* complex (AS-C) (García-Bellido and Santamaria, 1978; García-Bellido, 1979). He gave a masterful summary of the state of the knowledge and his work. It was mesmerizing to watch and listen to Antonio, his long, non-stop, staccato and sing-song delivery. Our very limited knowledge of fly genetics ill-prepared us for Antonio's sensory onslaught. We were totally overwhelmed by Antonio's talk and the genetic complexity of the *achaete-scute* complex. After we came back from Antonio's talk, we asked Alain what it was about. Alain assured us that although even he couldn't follow all of the subtleties, the subject of Antonio's talk was very important. Even though we could hardly understand Antonio's talk, it made quite an impression on us. We filed away in the back of our minds that "the *achaete-scute* complex is something important" and went on with our neurophysiology work.

In 1975, Antonio returned to Spain and Alain left Seymour's lab to take on his new position in Belgium. We stayed in Seymour's lab for two more years and worked on mutations affecting synaptic transmission. The mutant we spent most of our time on was *Shaker*. With the help of Mike Dennis at UCSF, we reached the conclusion that *Shaker* is likely to be a structural gene for a potassium channel (Jan

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0214-6282/97/$10.00

Printed in Spain

et al., 1977). One possibility then was to pursue the cloning of *Shaker*. At the time, cloning was in its infancy, we were not prepared to take on such a daunting task. Instead, we thought that some further training in hard core neurophysiology would be a good thing for us at that point. With the encouragement of Mike and Seymour, we went to Steve Kuffler's laboratory at Harvard Medical School. In Steve's lab, we stumbled upon the finding that a LHRH-like peptide is the transmitter for a mysterious, slow synaptic potential in the frog sympathetic ganglion (Jan *et al.,* 1979). This problem of peptide functioning as neurotransmitter kept us busy for the next three to four years, first in Steve's lab and then in our own little lab at UCSF after 1979.

In the early eighties, we linked up with Alain again. During the year that we were together in Seymour's lab, we interacted quite a lot and became good friends, even though we then worked on very different subjects. We thought it would be fun to do something together this time, and had two criteria for the choice of the project: (1) it should be something that we all found interesting and (2) it should not be the main research topic that any of us were already working on. For the next few years Alain, and later Alain and Christine Dambly-Chaudière, came to spend one to two months every year or two in San Francisco. We tried a few subjects, most having to do with neural development. During this period, Max Delbrück's famous "Principle of limited sloppiness" came into play. Because of our interest at the time in neuropeptides, we did a sort of Saturday afternoon experiment. For no profound reasons, we were simply curious in knowing if fly had Substance P-like peptides. One of us put antibodies on sections of the fly and the other one looked under the microscope. The entire fly nervous system lit up. This was odd, because neuropeptides tend to have very restricted distribution in the nervous system. We retraced our steps. It turned out that Lily had made a mistake. She picked up a wrong vial of antibody. We intended to use an antibody coupled to HRP. Instead, she grabbed an antibody raised against HRP. It was a lucky mistake. From that, we discovered that antibodies against HRP are very specific markers for all *Drosophila* neurons (Jan and Jan, 1982). This accidental finding provided us with a simple way to visualize the *Drosophila* nervous system.

Even with this neuronal marker and additional monoclonal antibodies we subsequently generated, the progress was very slow because we were unsure which part of the nervous system to focus on. In about 1985, we finally realized that the fly peripheral nervous system (PNS) is a good assay system for studying neural development. Alain, Christine and Rolf Bodmer worked out an accurate atlas of the embryonic PNS (Ghysen *et al.,* 1986, Bodmer and Jan, 1987). Then Alain and Christine made a crucial finding. Because of Antonio's influence, they have always had a strong interest in the AS-C. Naturally, the first mutants they looked at were ones of the AS-C. They saw a really striking phenotype. In AS-C mutants, one type of sense organ, the external sensory (es) organ, is completely missing whereas another type of sense organ, the chordotonal (ch) organ, is intact (Dambly-Chaudiére and Ghysen, 1987). This result is important because it provided the first evidence that a mutation can produce very clear-cut and cell type-specific phenotype. This result provided much needed encouragement that a genetic dissection of PNS development was feasible. As a result, we decided to start a systematic search for mutations affecting PNS development in 1985. Over the years, this approach has taught us some useful lessons about neural development (Ghysen *et al.*, 1993). This project is still going strong today.

Among the first group of mutants that came out of the PNS mutant screen was one on the second chromosome. In this mutant, the entire PNS is missing. To our great surprise, this mutant is an allele of the gene *daughterless (da)* (Caudy *et al.,* 1988a). *da* had previously been studied by Tom Cline for its role in sex determination (Cline, 1976). Initially, it was quite a puzzle that a gene would have an important function in two apparently unrelated biological processes. Help to resolve this puzzle came fairly quickly. In 1988, we cloned *da* and noticed that it had a sequence homology with *myc* and AS-C. Before we wrote the paper (Caudy *et al.,* 1988b), Lily went to a meeting at MIT. She happened to talk to David Baltimore at a reception and learned that his lab had just cloned a gene which encodes an immunoglobulin kappa chain binding protein E12/E47, which also has sequence homology with *myc* and AS-C. We then sent Baltimore the *da* sequence and it turned out that *da* is the homolog of E12/E47. Baltimore's lab had the remarkable insight to recognize a novel basic Helix-loop-Helix (bHLH) motif as a structural element for DNA binding and protein dimerization and that DA (the *da* gene product), Myc, AC and SC (gene products of *achaete* and *scute* in the AS-C), and MyoD all share this motif (Murre *et al.,* 1989a). This finding quickly led to the realization that DA and AC or SC normally form a heterodimer which binds DNA and regulates the transcription of downstream genes required for initiating neural development (Murre *et al.,* 1989b). In other words, DA and AS-C are positive regulators of neuronal development. DA is a ubiquitous factor whereas AS-C provides spatial information. The analysis of the expression pattern of the protein products of AS-C by J. Modolell and S. Carroll and their colleagues (Cubas *et al.,* 1991; Skeath and Carroll, 1991) led Alain and Christine to propose the important and very useful "proneural" concept (Ghysen and Dambly-Chaudiére, 1989).

Shortly after the realization that *da* and AS-C are positive bHLH regulators of neural development came the findings that *hairy* and *emc* belong to the same family as *da* and AS-C (Rushlow *et al.,* 1989; Ellis *et al.,* 1990; Garrell and Modolell, 1990). Earlier genetic work of Antonio already showed that *hairy* and *emc* are negative regulators of AS-C (Botas *et al*., 1982; Moscoso del Prado and García-Bellido, 1984). The molecular information provided a simple explanation of the mechanism. For example, Emc protein has the HLH motif but not the basic domain (Ellis *et al.,* 1990; Garrell and Modolell, 1990). It can bind DA or AC/SC and form dimers which are incapable of binding DNA. This would reduce the level of DNA-binding dimers of DA and AC/SC.

In parallel with the work on *Drosophila* neurogenesis, great progress was being made by H. Weintraub, E. Olsen and others in understanding vertebrate myogenesis. The way that bHLH factors are used to initiate development of those two systems turns out to be remarkably similar (Jan and Jan, 1993; Weintraub, 1993). The mystery of the connection of sex determination and neural development also largely disappeared. The bHLH proteins, as a group, work well as a genetic switch. Depending on the biological context, different downstream genes are the target of regulation of those bHLH proteins. In sex determination, it is *sex lethal*. In neural development, a different set of downstream genes are regulated by DA and AS-C.

Although the initial steps of neural development gradually became clear from these studies, there are still things amiss. In AS-C mutants, one type of sense organ, the chordotonal (ch) organ, is intact. Based on the way DA and AC/SC function, a simple

explanation is that there is an as yet unidentified gene X. Gene X most likely also encodes a bHLH protein and it should dimerize with DA to initiate ch organ development. With this assumption, we were able to find this missing gene and we called it *atonal*. Atonal does have all the properties we predicted (Jarman *et al.,* 1993). It is required for the initiation of ch organ development. An unexpected bonus was that *atonal* is also the proneural gene required for making the founder photoreceptor R8 in ommatedia assembly (Jarman *et al.,* 1994). This finding is satisfying. Because eye development depends solely on cell-cell interaction whereas es organ development also uses cell lineage mechanism, the mechanisms of eye development and es organ development were thought to be quite different. The finding that both systems use proneural mechanisms and Notch/Delta-mediated cell-cell interaction shows that there are more similarities than differences in their development. Although diversity underscores biology, we try to seek unifying themes whenever possible, perhaps because of our background in physics.

It is fascinating to observe how scientists choose their subjects. Many times throughout each scientist's life, they come to a branch point. Each choice may mean irreversibly going down a pathway. There is this recurring theme in Steven Jay Gould's "Wonderful Life":..replaying Life's tape (Gould, 1989). We cannot replay our life's tape. At each branch point, our choices are guided by our life history and serendipity. We don't know how our scientific career would have turned out if Antonio and Alain had not spent the 1974/1975 years at Caltech. Perhaps we would not have entered the field of Developmental Biology at all. We might have done something else. We will never know. Nevertheless, we were glad to have encountered Antonio during our impressionable age and to have been influenced repeatedly by him later on indirectly via Alain.

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