A note in passing: Walter Gehring and desert ants

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I was saddened to read of the passing of Professor Walter Gehring (1939–2014). One of his postdoctoral fellows, Michael Levine, wrote a personalized retrospective of the professional career of this famous developmental biologist, probably best known for the discovery of the homeobox and the development of useful tools such as the enhancer trap by the Gehring laboratory. An obituary was written by two of his former colleagues in Basel, Affolter and Müller (2014). I knew Walter via a shared interest in a biological problem that was not mentioned in either of these brief articles. We were both studying how animals sense and respond to elevated temperatures in the natural environment. In 1995, Walter along with Rüdiger Wehner published a paper in PNAS about heat shock protein (Hsp) synthesis and thermotolerance in one of the most heat-resistant animals known, the Saharan desert ant *Cataglyphis* (Gehring and Wehner 1995). Walter had been elected a foreign member of the US National Academy of Sciences and thus was able to contribute his own paper.

I have been a career-long reader of *Science* magazine, one of my favorite ways of keeping up with important work. I was engaged in the arcane activity of paging through the 14 April 1995 issue, opening to the Random Samples section, edited by Constance Holden. My eyes were immediately drawn to the title “Hot Ants”. The small photograph accompanying the article was captioned “Desert survivor. This ant can withstand blistering heat.” It showed an ant with unusually long legs in the process of moving spider-like across a patch of desert sand. The short article (Holden 1995) was a “teaser” for the PNAS paper by Gehring and Wehner. It went on to say that the ants could survive the 60 °C highs of the Sahara desert for

brief periods to feed on the “corpses” of less thermotolerant animals apparently by producing Hsps preemptively, in preparation to venture out of their underground nests into the desert heat. I had been studying Hsps and thermotolerance since the late 1970s, and I had obtained evidence supporting my “abnormal protein hypothesis” that Hsps are induced in cells that accumulate damaged proteins produced by a variety of stressors (Edington et al. 1989). I coined the term “proteotoxicity” for these events in a *Cell* review article (Hightower 1995), which Gehring cited in his paper. They also cited some of their own work in support of this hypothesis based on *Drosophila* mutations that produced chain-termination mutants in the actin gene, specifically in flight muscle (Hiromi et al. 1986).

The idea that the desert ant has evolved a special adaptation that allows it to somehow denature some proteins at will at relatively cool nest temperatures to activate the heat shock response seemed amazing indeed! We had shown earlier that cognate Hsc70, now known in humans as HSPA8 (Kampinga et al. 2009), has thermodynamic properties consistent with those of a sensor of temperature change, possibly a cellular thermometer protein (Leung et al. 1996).

I read the Gehring and Wehner paper with great interest and anticipation; Constance Holden’s “teaser” had done its job. It was a nicely written paper, and I was especially impressed with the way the authors zeroed in on what seemed to me to be the key findings relating to the molecular properties of the Hsp70 chaperones and what was known at the time about the physiology of the heat shock response and how it was deployed along with the animal-level response of acquired thermotolerance.

The experiments were carefully controlled using the temperate wood ant *Formica* and two species of *Drosophila* having substantially different Hsp induction temperatures. I did notice a technical limitation, the authors’ use of one-dimensional sodium dodecyl sulfate-polyacrylamide gel

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electrophoresis (SDS-PAGE). My graduate student Carol Norris had been working for several years on the application of two-dimensional high resolution SDS-PAGE to samples of various species of minnows from thermally distinct regions of the Sonoran Desert of subtropical Mexico. We had seen the essential importance of distinguishing between the constitutive (cognate) and inducible members of the Hsp70 family when comparing variation among species. It seemed likely even then that members of the Hsp70 family have similar but not identical functions. This, coupled with the fact that the one-dimensional gel patterns of *Formica* and *Cataglyphis* were qualitatively the same, suggested an alternative explanation.

I wrote a *Letter to Science* with the title “Desert Ants” that was published in the 9 June 1995 issue (Hightower 1995). I proposed that ants might have unusually high basal levels of Hsc70 that were augmented by inducible Hsp70 at elevated temperatures. At the cooler temperatures of their underground chambers, *Cataglyphis* thus would have on-board extra capacity for dealing with the extreme heat of the desert surface. Perhaps its inducible response, triggered by heat-denatured proteins, was not very different from *Formica*, and indeed, the gel patterns of polypeptides from the two species looked that way. I played upon a quotation from Walter, included in the article, about the possible anticipatory acquisition of thermotolerance, “We are not clever enough to think of this,” “but the ants were.” I suggested that “Perhaps desert ants are smart enough to return to the nests before their proteins melt down...,” and that this was less amazing than the mental image of ants turning on their heat shock genes in anticipation of “hotfooting it” across the desert sands, but interesting nonetheless. Apparently, the editors agreed, and they published my letter along with the same photo of the long-legged ant, this time with the caption “Hotfooting it: How does the ant, *Cataglyphis bombycina* survive the desert heat?” Never underestimate the power of a turn of phrase! This has been my only letter accepted by the editors of *Science*. Walter and I corresponded briefly thereafter about our studies and our shared interest in the physiology of the heat shock response.

The *Science* letter also provided me the opportunity to put in a reference to Carol Norris White’s superb dissertation research on the heat shock responses of small fish in the genus *Poeciliopsis* (White et al. 1994; Norris et al. 1995). I was later made a Fellow of the American Association for the Advancement of Science based largely on the papers that flowed from Carol’s Ph.D. thesis, an honor I will always share with her. I never had the privilege of meeting Walter Gehring, but based on Michael Levine’s description of the man as an unusual mix of the professorial and authoritative combined with folksy and unaffected mannerisms, along with the inclination to laugh and joke, I would have liked Walter.

I was stimulated by Michael Levine’s retrospective to read again Walter’s PNAS paper and reminisce about our brief intersection of minds almost 20 years ago. Suddenly, there it was, the answer to the thermal adaption of *Cataglyphis*, in Fig. 1f (Gehring and Wehner 1995), a panel comparing the temperature profile of HSP70 synthesis in *C. bombycin* and *F. polyctena*! In their Discussion section, Gehring and Wehner had drawn upon information about heat-resistant bacteria that have adapted biochemically to high temperatures by evolving enzymes and other proteins with high thermal denaturation temperatures. Reasoning by analogy, they proposed that the proteins of *Cataglyphis* might be more stable than those of insects living in more temperate environments. They then concluded that if this reasoning is correct, the temperature at which the response is triggered by denatured proteins should be higher in *Cataglyphis* than in *Formica*. They pointed out that the data in Fig. 1f show the opposite is true. The problem is that this is not a good analogy, so their reasoning, borrowed from bacterial adaption to life at high temperature, “had got a bloomer in it,” one of my favorite Francis Crickisms. Bacteria have adapted to life at a fairly narrow range of high temperatures for each strain and have evolved enzymes that function optimally at these temperatures. The important difference between these ants and thermophilic bacteria in this context is that even desert ants spend most of their time metabolizing at the cooler nest temperatures and only spend brief periods of time at highly elevated temperatures. Thus, they need enzymes that function optimally at temperatures of say 20–25 °C along with some way to protect their proteins at the high temperatures briefly encountered on the surface of the Saharan desert. Affolter and Müller mentioned in their article that “Walter always had a somewhat simplistic idea of things. Although he knew about the complexity of life, he favored simple hypotheses.” He certainly had his share of successes using this approach, and certainly more was known at the mechanistic level about bacteria than any other cell so it was reasonable to try any analogy in that direction. We now know that the answers bend in the direction of biological complexity for this problem, which we are still far from fully understanding.

So, what are the additional biochemical adaptations to heat beyond the temperate *Formica* that *Cataglyphis* brings to the table? Gehring and Wehner counted both of them as the most important findings of the study. How would the responses that were responsible of the kinetics in Fig. 1f of their paper play out in ants in the Saharan desert? First, as the temperature of the nest increases slightly, or perhaps the ants move into a tunnel only a short distance from the nest and pause, the ants’ heat shock response is activated, and this occurs about 4 °C before the *Formica* response is activated. Second, as the temperature increases beyond the critical temperature above which *Formica* cannot sustain protein synthesis, about 39 °C, *Cataglyphis* continues to synthesize proteins, especially Hsps, through at least 4 additional degrees Celsius. Both of

these adaptations would contribute to *C. bombycina* having a critical thermal maximum of 53.6 °C whereas *F. polycтена*'s is 46.8 °C (the temperature at which the animal is no longer capable of proper locomotion).

The one-dimensional gels indicate that *Cataglyphis* and *Formica* share the ability to produce substantial basal levels of Hsps at normal nest temperatures. No doubt this was useful in the evolution of additional biochemical adaptations in *Cataglyphis* allowing survival at insanely high temperatures for animals. If anything, I would expect some of the proteins of *Cataglyphis*, perhaps even a thermometer or sensor protein, to be more thermolabile than those of *Formica*, triggering the induction of the *Cataglyphis* heat shock response at a lower temperature. The second adaptation noted by Gehring and Wehner, increased heat resistance of the *Cataglyphis* protein synthetic apparatus would be key as well. Once triggered in or near their underground nests, Hsps will accumulate to elevated levels even while the ants are in the hot sun and after they return to their nests, minimizing thermal damage and allowing a more rapid recovery. Given the substantial body of results that has accumulated over the past 20 years supporting the concept of proteotoxicity as a trigger for the heat shock response, I think we can in hindsight conclude with confidence that adaptation of acquired thermotolerance and not evolution of more heat-resistant proteins is what distinguishes these two species.

Walter is much more broadly known in the heat shock field for his collaborations beginning with Alfred Tissières, the pioneering discoverer of the heat shock proteins, on the cloning of *Drosophila* heat shock genes (Schedl et al. 1978). I wish that I could communicate with Walter to enjoy his thoughts on these ideas the way we did 20 years ago. I am certain that he would have sharpened and expanded them, probably oversimplified them, and suggested elegant experiments to test them. We are fortunate that he left us a legacy of

many papers including this one (Gehring and Wehner 1995), published many years ago, which are still useful today. The enduring papers are that way!

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