

**PASPCR Commentary:
Spotting the spotted
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We scientists always feel compelled to provide reasons for why our research is especially worthwhile and interesting. In fact, there seems to be a reason for all science and a science for all reasons. So, the humorist may quip that *everything* is interesting and that we might just as well do away with the requirement to provide specific rationales for our work. But if specific rationales were not that important, is there something more central that makes us get up early in the morning and work until late at night? Is there some other key feature that makes science a passion rather than a profession? I believe there is. It is the “A-to-Z” strategy, a strategy which we share with few others—farmers, perhaps, and artists. Like artists decide what to create, we have the luxury to decide which problems to tackle. Like farmers sow and till the field, we perform our experiments. And like artists exhibit their work and farmers harvest the fruits and take them to the market, we collect the results and publish our reports. Granted, the architect designs the structure, draws the plans, and may even finance and sell the building, but she does not herself lay brick upon brick. The librarian creatively arranges the books, preserves them, and presents them to the visitors, but he does not himself write those books. As scientists, we do it all...well, at least as long as we have not yet been promoted to group leaders, and we treasure this freedom.

Now, after these lofty thoughts, it may come as a surprise that my own career seems to have been guided not by deep philosophical reflections but simply by an attraction to the letter “M”. That’s not even counting that I am an MD and am working with mice. As a virologist, from the nineteen-seventies until the early nineteen-nineties, I worked on a gene called *Mx* (more on this below). Later, there was an interlude in work on *Meox*, and then came *Mitf*. Moreover, at the same time, all my girl friends had M’s in their names. So, perhaps, my life’s rationale is just a quirk of the mind to prefer M’s?

All these M’s were important, though, as they have profoundly influenced my thinking. I entered science fascinated by the observation that some individuals are more

resistant than others to the same viral infection. We know, of course, about the benefits of the adaptive immune system, but that is not what I am talking about here. I am talking about an underlying *genetic* resistance to specific viruses, for which there are excellent animal models. One particularly intriguing case of such a genetic resistance was discovered and studied by my thesis advisor and friend Jean Lindenmann from the University of Zürich, Switzerland, who also discovered interferon along with his late colleague, Alick Isaac, when both worked at Mill Hill, London, UK. In 1962, Lindenmann observed, by chance, that mice belonging to an uncommon inbred strain called A2G survived infection with influenza viruses at doses so high that they would kill 10,000 or even 100,000 “normal” laboratory mice. He found that this resistance was due to the action of a single autosomal-dominant gene that he called *Mx* (for resistance to “orthomyxoviruses”, now synonymous with influenza viruses), and that it covers all influenza A and B viruses but not other RNA or DNA viruses.

For a long time, there were two prevailing thoughts to explain this phenomenon: Because resistance was virus-specific, we thought first that A2G mice must have a particularly efficient immune reaction against influenza, and second we thought that interferon could not be involved, for interferon was not virus-specific and anyway reached only very low titers in the resistant mice. But then came the “paradigm shift”, that most gratifying experience in any scientist’s life: interferon was involved, after all. What all previous approaches to curb the adaptive or innate immune system failed to achieve was readily accomplished by antibodies to interferon: resistance was broken.

After this discovery, pioneered principally by Otto Haller, we went back to science-as-usual. What followed was the discovery of the MX protein by Michel Horisberger, the cloning of the interferon-inducible *Mx* gene by Peter Staeheli working in collaboration with Otto Haller and Charles Weissmann, and the finding that *Mx* encodes a large GTPase capable of specifically interfering with influenza virus replication. And here is how it works: Early on, when just a few cells are infected in A2G mice, interferon is made locally. *Mx* is then induced in cells in the immediate vicinity, and viral spread is aborted. In susceptible mice (that is, all other laboratory strains besides A2G and another uncommon strain, SL/NiA), initial viral replication is similar. Interferon and *Mx* are induced just as well, but the *Mx* gene has crippling mutations and so the virus can

spread unimpeded. Important, however, as long as there is no infection, the genetically susceptible mice are as normal as the genetically resistant ones.

So I learned some critical lessons from these findings: there is virtue in chance observations; genes are not genes unto themselves, but interact with the environment; seemingly non-specific actions (as that of interferon against many viruses) can be the sum of multiple specific actions; things that can easily be overlooked (small amounts of interferon, few cells expressing *Mx*), when present at the right time and place, can defy death or disease; and standard knockout mice, say in a gene “X”, are, in fact, at least double knockouts, in “X” and “*Mx*”, solely to indicate this fact. All this impressed me intensely and was responsible for my decision to give up medicine for good and become a full-time experimentalist.

After 20 years of *Mx* research, though, it was time to move on. The new cause of excitement was another chance observation: an insertional mutation in a transgenic mouse that made the mouse white, microphthalmic, and deaf. At the time, the cloning of a gene responsible for the phenotype associated with a transgenic insertion was not easy, but a combination of genius and luck helped Colin Hodgkinson, a postdoc in the lab from the UK, to come up with the sequence of the now famous gene that underlies the *microphthalmia* mutation. And so a new field was born, the field of *Mitf*.

Little did we know at the time that *Mitf* has its fingers in virtually every aspect of the biology of vertebrate melanin-bearing pigment cells. It specifies the precursor cells; it helps them survive when they are derived from the neural crest, or at least keep them on track developmentally when they are derived from the optic neuroepithelium; it regulates their proliferation and differentiation; it controls their renewal from stem cells; it even plays roles during malignant transformation as a “lineage addiction oncogene”. It does all this, or at least most of it, through its capacity to bind particular nucleotide sequences, so called E-boxes, in enhancers/promoters of target genes whose expression it regulates. But the days when we thought of MITF as a transcription factor specific to pigment cells and perhaps a few other cell types are long gone. MITF is found everywhere: in the kidney, in the uterus, and in fibroblasts, though the protein levels and isoforms differ from cell type to cell type. The gene turned out to be fairly complex, with at least nine promoters and seventeen exons, and a few “subexons”, giving rise to a family of splice

variants and proteins subject to multiple post-translational modifications, including phosphorylation, ubiquitination, sumoylation, acetylation, and more. And then there are myriad mutant alleles from fish to man, and relatives in worms, flies, and urochordates, just to keep the student of molecular genetics busy. Our task now is to make sense of all this complexity: Are the transcriptional isoforms of *Mitf* regulated separately or coordinately? Do the various isoform-specific aminotermini have any distinct roles during development, regeneration, and malignant transformation? Is there a correlation between splicing and biological activity? When and where do the post-translational modifications take place, and are they relevant for the biology associated with *Mitf*? Fortunately, the field has become big and accommodates both small groups like mine and large groups mostly devoted to cancer. *Mitf*, we can safely assume, is now a multi-million dollar enterprise. On the one hand this fills me with pride. On the other, I long for the days when we could still make a living simply by spotting the spotted.

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Further readings

LINDENMANN, J., 1962 Resistance of mice to mouse-adapted influenza A virus. *Virology* 16: 203-204.

HALLER, O., H. ARNHEITER, J. LINDENMANN and I. GRESSER, 1980 Host gene influences sensitivity to interferon action selectively for influenza virus. *Nature* 283: 660-662.

HORISBERGER, M. A., P. STAEHELI and O. HALLER, 1983 Interferon induces a unique protein in mouse cells bearing a gene for resistance to influenza virus. *Proceedings of the National Academy of Sciences of the United States of America* 80: 1910-1914.

STAEHELI, P., O. HALLER, W. BOLL, J. LINDENMANN and C. WEISSMANN, 1986 Mx protein: constitutive expression in 3T3 cells transformed with cloned Mx cDNA confers selective resistance to influenza virus. *Cell* 44: 147-158.

ARNHEITER, H., S. SKUNTZ, M. NOTEBORN, S. CHANG and E. MEIER, 1990 Transgenic mice with intracellular immunity to influenza virus. *Cell* 62: 51-61.

HODGKINSON, C. A., K. J. MOORE, A. NAKAYAMA, E. STEINGRIMSSON, N. G. COPELAND, N. A. JENKINS and H. ARNHEITER, 1993 Mutations at the mouse microphthalmia locus

- are associated with defects in a gene encoding a novel basic-helix-loop-helix-zipper protein. *Cell* 74: 395-404.
- ARNHEITER, H., M. FRESE, R. KAMBADUR, E. MEIER and O. HALLER, 1996 Mx transgenic mice—animal models of health. *Current Topics in Microbiology and Immunology* 206: 119-147.
- ARNHEITER, H., L. HOU, M. T. T. NGUYEN, K. BISMUTH, T. CSERMELY *et al.*, 2006 Mitf—A matter of life and death for the developing melanocyte in *From Melanocytes to Malignant Melanoma*, edited by V. HEARING and S. P. L. LEONG. Humana Press, Totowa, NJ.
- BHARTI, K., M. T. NGUYEN, S. SKUNTZ, S. BERTUZZI and H. ARNHEITER, 2006 The other pigment cell: specification and development of the pigmented epithelium of the vertebrate eye. *Pigment Cell Research* 19: 380-394.