© 2018. Published by The Company of Biologists Ltd | Development (2018) 145, dev164616. doi:10.1242/dev.164616

OBITUARY

Obituary: Denise Barlow (1950-2017)

Anne C. Ferguson-Smith[1,*] and Marisa S. Bartolomei[2]

ABSTRACT

Anne Ferguson-Smith and Marisa Bartolomei look back at the life and science of Denise Barlow, a pioneer in genomic imprinting and epigenetics.

Image courtesy of Anita Ender (©CeMM/Leitner)

On October 21st 2017, the imprinting and epigenetics community lost a pioneer. Denise Barlow spent the majority of her independent career identifying and studying the mechanistic regulation of the *Igf2r* imprinted gene cluster. In the process, she demonstrated how imprinted genes could serve as a paradigm for epigenetic gene regulation. She also identified one of the first long non-coding (lnc) regulatory RNAs, performing elegant experiments to elucidate how lncRNAs could regulate genes in *cis*. Denise retired in 2015 as a principal investigator at the Center for Molecular Medicine (CeMM) in Vienna, Austria.

Denise Barlow was born on January 31st 1950, in Harrogate, UK. She worked as a state-registered nurse in the UK before switching careers and attending Reading University, where she discovered her excitement for developmental biology. Denise carried out her PhD thesis work at Warwick University with Professor Derek Burke, investigating when a mouse embryo developed an interferon response to viral infections. Her passion for development drove her to perform her initial postdoctoral research stint at the Imperial Cancer Research Fund (ICRF) Mill Hill Laboratories in London, where she was Brigid Hogan's first postdoctoral fellow (1981-1985). There, she worked on isolating genes expressed early in mouse development, before many of the methods that we now take for granted were available. She then met Hans Lehrach who told her about the molecular genetic tools he was developing to map and isolate mouse genes. She went to the Lehrach lab at the European Molecular Biology Laboratory (EMBL) in Heidelberg on an EMBO long-term fellowship (1985-1988) and learned many of these new technologies, including her favourite, pulsed field gel electrophoresis, which allowed separation of very large chromosomal fragments. In 1988, she took her first group leader position at the Institute of Molecular Pathology (IMP) in Vienna. Denise then spent 4 years as group leader at the Netherlands Cancer Institute in Amsterdam before returning to Austria, first as a Department Head at the OAW Institute of Molecular Biology in Salzburg (which closed in 2003) and then as a CeMM Principal Investigator until she retired.

Denise made many contributions to our understanding of mammalian molecular genetics, but her studies on the epigenetic control of genome function and, in particular, her contributions to genomic imprinting were the most estimable and can best be illustrated in three remarkable discoveries. First, in 1991, Denise was the first to identify an endogenous imprinted gene, insulin-like growth factor receptor 2 (*Igf2r*). Using mouse genetic models which exhibited phenotypes with parental origin effects in their patterns of inheritance that mapped to chromosome 17, Denise applied the systematic physical mapping approaches that she had learned in Heidelberg first to narrow down the region of interest on mouse chromosome 17 and then to assess the expression of candidate genes. The *T hairpin* (*Thp*) mouse, which harboured a deletion on chromosome 17, was particularly valuable for these experiments. When maternally inherited, this deletion resulted in embryonic lethality but, by contrast, resulted in viable offspring when inherited from the father. Denise showed that, whereas other transcripts mapping to the deletion were expressed in all *Thp* heterozygous embryos regardless of the parental origin of the deletion, *Igf2r* was only expressed if an intact maternal chromosome was present (Barlow et al., 1991). When the deletion was maternally inherited no transcripts were evident. This indicated that the gene was repressed on the paternally inherited chromosome and expressed from the maternally inherited one. This functional non-equivalence of the parental genomes in offspring – now termed genomic imprinting – had been recognised through sophisticated mouse breeding observations and through embryo manipulation experiments, but Denise's identification of *Igf2r* as the first imprinted gene was pivotal in establishing imprinting as a valuable model for the study of the epigenetic control of transcription. That discovery paved the way for future experiments on epigenetic inheritance, and provided data upon which hypotheses concerning the evolution of the bizarre

[1]University of Cambridge, Department of Genetics, Downing Street, Cambridge CB2 3EH, UK. [2]Department of Cell & Developmental Biology, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104, USA.

*Author for correspondence (afsmith@gen.cam.ac.uk)

A.C.F., 0000-0002-7608-5894; M.S.B., 0000-0001-9410-5222

Development (2018) 145, dev164616. doi:10.1242/dev.164616

process of genomic imprinting could be generated and debated – debates in which Denise was an active contributor.

In a second major advance, Denise then went on to determine the epigenetic mechanism that distinguishes the two genetically identical alleles of *Igf2r* in a normal developing mouse embryo, allowing one to be activated and the other to be repressed. In 1993, her team identified two regions of *Igf2r* that were differentially DNA methylated on the two parentally inherited chromosomes – one region located at the *Igf2r* promoter and the other in the second intron (Stöger et al., 1993). She showed that of these differentially methylated regions (DMRs), the intronic one was inherited from the germline as a DMR, established as methylated in the female germline and inherited in an unmethylated state from sperm. By contrast, the promoter DMR acquired its differential methylation late in embryonic development, long after fertilisation. The methylation at the promoter was acquired on the paternal allele after it had become repressed, with the active maternal promoter allele remaining unmethylated. Importantly, she hypothesized correctly that it was the intronic germline DMR that was the imprinting control element, since its deletion resulted in loss of imprinting (Wutz et al., 1997). Specifically, a paternally inherited deletion of the unmethylated intronic region resulted in activation of the normally repressed *Igf2r* from that chromosome. By contrast, upon deletion of the methylated maternal element, nothing happened to the active *Igf2r* copy. Thus, it was the unmethylated element in the paternally inherited intron that was essential for the repression of the paternally inherited *Igf2r* allele. Barlow's methylation analysis was paradigm shifting for three reasons. First, it suggested that germline DNA methylation is the heritable memory of parental origin that regulates imprinting, a hypothesis that she later went on to prove using targeted mutagenesis. Second, and surprisingly, it showed that a methylation imprint could be present on the chromosome that expressed the imprinted gene (Stöger et al., 1993). Finally, it emphasized that promoter methylation could be a consequence of transcriptional repression and not necessarily its cause.

This pivotal epigenetic analysis raised the question of how the intronic germline DMR might be essential for the repression of *Igf2r* in *cis*. Denise figured this out in a remarkable set of papers. These initiated with the discovery of an imprinted antisense lncRNA (named *Airn*) expressed from the intronic DMR on the unmethylated paternally inherited allele (Wutz et al., 1997). Subsequently, using very elegant transcript truncation experiments, she proved that expression of this lncRNA, regulated by the intronic DMR, was required for *Igf2r* imprinting (Sleutels et al., 2002). Finally, the team showed that the act of antisense expression from the paternally inherited intron, up to and overlapping the *Igf2r* promoter, confers transcriptional interference occluding the promoter on the paternally inherited chromosome and preventing RNA polymerase II recruitment, resulting in repression of the paternally inherited *Igf2r* (Latos et al., 2012). Denise's systematic deciphering of the mechanism of *Igf2r* imprinting has even wider implications given the large number of lncRNAs in the mammalian genome. This simple epigenetically controlled mechanism of *cis*-acting regulation via transcriptional interference reflects a more widespread phenomenon at non-imprinted loci (Koerner et al., 2009).

Although Denise never sought awards, just answers to scientific questions, she was the recipient of numerous honours. She became an EMBO member in 1995, and was a member of the EMBO Science & Society Committee between 1999 and 2003 (chairing this committee from 2002-2003). In 2003, Denise was awarded Honorary Professor of Genetics at Vienna University, and received both the Erwin Schrödinger Prize of the Austrian Academy of Science and the EMBO/EMBL Austrian Chapter Achievement Award Medal for lifetime achievements in 2014.

Denise was truly unique – a passionate scientist, a superb geneticist, an innovator and a fearless experimentalist who eagerly embraced new technology. She was rigorous in her science, never cut corners no matter how hard an experiment turned out to be and always included thoughtful controls. Most of all, Denise wanted to understand how things worked. She always thought of the alternative, out of the box explanation, which enabled her to make many breakthroughs, although it wasn't always easy to convince her critics. If you didn't know Denise, and you both attended the same meeting, it was surely within the first few lectures that you came to know her. She would ask smart and thoughtful questions after many talks, sometimes curious and sometimes combative. If you were giving an imprinting talk (or writing a paper that she might review), you definitely wanted to avoid the terms 'maternally imprinted' and 'paternally imprinted'. These terms were often assumed to imply the transcriptional state of an allele but an imprint could be a positive or negative regulator of the imprinted gene – we agreed with Denise that the most accurate terminology is 'expressed or repressed allele'. If you had a slide with a mistake or a missing reference, she would make sure that your error was corrected, and you would not present the erroneous slide again.

One of Denise's most notable traits was her strong sense of fairness and inclusivity. In her trainees, she instilled skills in critical evaluation of their own and others' work, a lack of compromise in their experimental design and caution in the interpretation of their data. She was a strong advocate for women and would critique the proportion of female speakers at conferences. She realised the importance of visibility to these female scientists; she wanted to highlight their work, not only for the women themselves, but also for the conferences' young scientists, who needed to see the great science of impressive women.

Denise Barlow was publishing right up to the end. With her death, the molecular genetics and epigenetics field has lost one of its most rigorous and productive scientists, and many of us have lost a highly respected colleague and friend. Her uncompromising pursuit of the scientific truth has changed the way we think about DNA methylation, non-coding RNAs, and the relationships between epigenetic states and transcription. She has left a lasting imprint.

Acknowledgements

We are grateful to Denise's colleagues who shared their memories and provided anecdotes of her life and science that shaped this obituary. Particular thanks to Paulina Latos and Reinhard Stöger for feedback on the text and to Anita Ender (CeMM) for the photograph (copyright CeMM/Leitner).

References

Barlow, D. P., Stöger, R., Herrmann, B. G., Saito, K. and Schweifer, N. (1991). The mouse insulin-like growth factor type-2 receptor is imprinted and closely linked to the Tme locus. *Nature* **349**, 84-87.

Koerner, M. V., Pauler, F. M., Huang, R. and Barlow, D. P. (2009). The function of non-coding RNAs in genomic imprinting. *Development* **136**, 1771-1783.

Latos, P. A., Pauler, F. M., Koerner, M. V., Şenergin, H. B., Hudson, Q. J., Stocsits, R. R., Allhoff, W., Stricker, S. H., Klement, R. M., Warczok, K. E. et al. (2012). Airn transcriptional overlap, but not its lncRNA products, induces imprinted Igf2 silencing. *Science* **338**, 1469-1472.

Sleutels, F., Zwart, R. and Barlow, D. P. (2002). The non-coding Air RNA is required for silencing autosomal imprinted genes. *Nature* **415**, 810-813.

Stöger, R., Kubicka, P., Liu, C. G., Kafri, T., Razin, A., Cedar, H. and Barlow, D. P. (1993). Maternal-specific methylation of the imprinted mouse Igf2r locus identifies the expressed locus as carrying the imprinting signal. *Cell* **73**, 61-71.

Wutz, A., Smrzka, O. W., Schweifer, N., Schellander, K., Wagner, E. F. and Barlow, D. P. (1997). Imprinted expression of the Igf2r gene depends on an intronic CpG island. *Nature* **389**, 745-749.