FINIS ORIGINE PENDET

This Latin motto of Phillips Academy Andover, translated "The end depends on the beginning," captures the illustrated message that I have created for colleagues who have joined Alexandra in both her family life and her professional career. This message is composed along four interconnected signposts that are important guides in our family: Do Your Homework; Do Good Work; Just Connect; and Behold Beauty. Alexandra's version of this message moves from Family to Community of Science.

Our three offspring were born over a narrow span of three and a half years. Busy raising these youngsters, Alexandra set aside her research career over the seven years until each of our children were in primary school. At the moment when all three were "deposited" in first grade of the French system, Alex joined into a serious research program in mouse biology led by François Jacob at the Institut Pasteur. A major figure in that program was Jean-Louis Guénet. Connecting with and through Jean-Louis has remained important to Alexandra's and Bill's family life and scientific research.

Do Your Homework

Strolling through Tuileries Garden, August 1975

The effort that Alexandra had invested as a stay-at-home mother of these three children gave them both self-confidence and the mutual support to survive being immersed in a new language and culture. Further along in this essay we shall see these three in 2021 and understand Alexandra's view that her family is her most enduring contribution.

The Jacob research group included mature researchers in several disparate fields of mammalian biology, including the genetics of the laboratory mouse. Alexandra joined with Jean-Louis Guénet to develop her command over the

intricacies of experimental investigations with the mouse. She was a natural in this research, stemming from her enjoyment of mice as an eight-year-old, developing a "mouse circus" in the basement of her family's apartment in New York City. From Paris to Madison, Alexandra conveyed the new methods in the molecular genetics of the mouse, and her personal connections with Jean-Louis and his French colleagues. She was determined to test our lab's proposal to develop point mutagenesis of the mouse germline. Combined by progress in DNA sequencing, only dimly perceived in the mid-1970s, point mutagenesis enables the analysis of particular biological processes in mammals at molecular resolution. From 1978, Alexandra worked toward this goal with McArdle's Research Specialist Linda Clipson, bringing together the necessary attention to detail, understanding of the mouse and its genetics, and command over large sets of data and presentation of these data. Starting in the 1980s, Alexandra, Bill and Linda were joined in McArdle by interested undergraduate and graduate students[a], research specialists[b], postdoctoral fellows[c], and broadly interested members of our subgroup developing the genetics of the protist Physarum[d].

A large departmental grant supported the mouse facility in McArdle. It had been created from 1940 by the breakthrough research in chemical carcinogenesis by James and Elizabeth Miller, Roswell Boutwell, Charles Heidelberger, and Van Potter. The research emerging from mutagenesis of the mouse germline was feasible thanks

Do Good Work

Alex in the mouse colony, 1982

[a] Tom King, Karen Gould, Cindy Luongo, Camille Connelly, Derek Symula, Alex Shoemaker, Bob Cormier, Amy Lillich, Kevin Haigis, Larry Kwong, Jake Prunuske, Jesse Waggoner, Amy Irving, Elisa Dunkin, Jennifer Pleiman, and Eric Toraason

[b] Natalie Borenstein, Darren Katzung, Cheri Pasch, Dawn Albrecht, and Kathy Krentz

[c] Larry Johnson, Amy Moser, Dave McDonald, Andrea Bilger, Anita Merritt, Rich Halberg, Xiaodi Chen, Bill Ehrhardt, Andy Thliveris, and Jim Amos-Landgraf

[d] Tim Burland, Tim Schedl, and Lila Solnica. Lila went on as a postdoc in Boston to demonstrate that ENU is a germline mutagen superior to EMS, the standard of Drosophila geneticists [Genetics 1994; 136:1401-1420].

to this McArdle facility. The facility was expanded through a McArdle grant that included mouse mutagenesis, submitted by James Miller in 1977. In 1979, Bill Russell at Oak Ridge [1], identified the directly acting nitrosamide ethylnitrosourea (ENU) as the most effective point mutagen in the germline of the mouse. He too drew from the chemical carcinogenesis field – Ekkehart Vogel, PD Lawley, and Peter Brookes, a sabbatical visitor to Heidelberger in McArdle.

The new mouse mutagenesis program in McArdle was linked in the 1980s to investigators worldwide[e] by the Mouse Mutagenesis Memo (MMM) coordinated by Alexandra and Bill Dove, as reported by Jean-Louis Guénet [2]. Guénet became instrumental in promoting the worldwide sharing of the basic methods that remain necessary to make mouse germline mutagenesis feasible.

Successes in mutant isolation from several of these sites, combined with the full sequencing of the mouse genome [3] created a new opportunity to analyze a biological process at molecular resolution. When a process is affected by a genetic difference as simple as a point mutation, the investigator learns that the product of the affected gene is involved in the process of interest. A worldwide research "industry" has emerged with the laboratory mouse, using the phenotype-driven strategy to discover molecules for which an individual change affects a biological process of interest.

Making mutagenesis feasible for a single investigator in a university setting, a key question is: How many mice must be screened if only one gene is involved in the process of interest? Russell and his colleagues in the Oak Ridge National Laboratory observed that ENU-induced mutations arise in the range of one per gene per fifteen hundred mice [1]. Alexandra joined Jean-Louis and others in reporting the induction by ENU of a series of mutations in proximal mouse chromosome 17, the large region containing the "T-locus" that developmental biologists knew to be important in mouse development. The region proved to be sufficiently large that 12 independent ENU-induced mutations affecting embryonic

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[e] Draft list of the active MMM contributors - McArdle (Alexandra Shedlovsky and Bill Dove); Kansas State (Vernon Bode, Monica Justice and Dave McDonald); Roswell Park (Verne Chapman and Ken Paigen); Research Triangle Park North Carolina (Susan Lewis); Texas Southwestern (Kristen Fischer-Lindahl and Bruce Beutler); Oak Ridge (Liane Russell); JAX (Wayne Fraankel; Tibby Rusell); Pasteur (Jean-Louis Guénet, Phil Avner, and Xavier Mongatutelli; Harwell (Jo Peters and Mary Lyon).

development were found - at a frequency around one per 20 mice screened. Their 1986 publication [4] continues to be cited - 73 times overall, as recently as in 2020.

While developing genetic markers for the *T*-region in a cross between two disparate strains of the mouse, Alexandra's sharp eye for mutant mice led to the discovery of a mutant lacking a tail, bearing a dominant mutation also mapping to proximal chromosome 17 — T^{Wis} [5]. Tom King, working with Natalie Borenstein in the lab and Bernard Herrmann and Hans Lehrach in Germany, established the successful molecular identification of the developmental gene *Brachyury.* A key to this success was showing that the mutation in T^{Wis} is an intragenic insertion of a retrotransposon[f]. By contrast, other mutant alleles at the *T*-locus are multigenic deletions.

Identifying the *Brachyury* gene at the molecular level continues to be important in understanding polarity in embryonic development. The report by Herrmann and colleagues in 1990 [6] has been cited in 687 publications, including ten times as late as 2021. The embryologist Virginia Papaioannou has the T^{wis} allele to study mouse embryos inactivated in Brachury function [7].

Alexandra and her colleagues have shown that an efficient mutagenesis program in a university setting is feasible, given both a sharp eye and efficient mutagenesis. The efficiency of the ENU mutagenesis program in McArdle was illustrated further by the discovery of the Min mouse by Amy Moser. Amy, trained at Michigan in the laboratory of Bob Erickson (whom we had befriended while working with Jean-Louis), is alert to many facets of mammalian biology. Amy was initially attracted to a family in the mutagenesis program that showed dominant transmission of a circling behavior. Following this family through multiple generations, she showed that it also carried a second mutation, independent of that causing the circling behavior. This mutation showed dominant transmission of adult-onset anemia causing pale feet, noticed when Amy clipped the toes to mark mice individually for the massive databases of the ENU program. When animals showing the "Pale foot" phenotype were analyzed by Amy and McArdle's

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[f] It is plausible that the T^{Wis} mutation arose by a genetic instability process known in the genetics of Drosophila as "hybrid dysgenesis". See Engels WR, Extrachromosomal control of mutability in Drosophila melanogaster, PNAS, 1979; 76(8):4011-5.

pathologist Henry Pitot, the affected mice were shown to carry multiple adenomas throughout the intestine. The causative dominant mutation was therefore named *Multiple intestinal neoplasia — Min.* The molecular analysis of mammalian genomes enabled collaborators in the laboratory of Ken Kinzler and Bert Vogelstein at Johns Hopkins to identify *Min* as a T to A point mutation that truncated the large polypeptide product of the mouse homolog of the human *APC* gene [8]. The APC protein is interpreted as a function necessary for early development [9] and as a "gatekeeper" that controls colonic adenomatosis in humans.

The inbred C57BL/6J $Apc^{Min/+}$ strain has been made broadly available to researchers in much of the world through The Jackson Laboratory (JAX). Ken Paigen, Director of (JAX) in the 1990s, was instrumental at bringing into the mouse genetics community ENU-induced mutants such as the Min strains. Ken and his wife Beverley were long-term friends of the Dove family from summer visits to Mt. Desert Island and of Jean-Louis from a sabbatical at the Pasteur. While working with Richard Gardner's embryology group in Oxford, Alexandra and Bill were able to introduce the Min strain into England, overcoming the quarantine imposed to prevent rabies transmission. Since its publication in 1990, research with the Min strain worldwide has been reported 1734 times, including 82 in 2021. These reports include 92 involving British laboratories, many funded by Cancer Research UK, a national resource for research on cancer in Britain that has inherited the Min mouse colony whose importation had been facilitated by Gardner in 1991.

Final success in a mutagenesis program often emerges when focused on a particular biological process. A fruitful example is the program of Bruce Beutler on natural resistance and the genetic network involving the Toll receptor. For Beutler's comprehensive analysis of the power of ENU mutagenesis, see his review [10]. Jean-Louis provided early advice to Beutler on the methods of ENU mutagenesis. Vernon Bode at Kansas State, who shared with Bill a background in the genetics of phage lambda during their time as postdocs in Stanford, independently developed an ENU mutagenesis program, focused on the metabolic disease Phenylketonuria. He too strengthened his command over mouse genetics on sabbatical leave with Jean-Louis. Tragically, in 1986 a severe stroke ended his active research career directing his program. Vernon's doctoral students Monica

Justice and Dave McDonald have carried forward the torch lit by Vernon. Dave collaborated with Alexandra in a concentrated study of hyperphenylalaninemia (HPH) caused by ENU-induced mutations in the mouse.

Focusing on the HPH phenotype, Dave, Alexandra and their colleagues reported the isolation, mapping and molecular characterization of numerous ENU-induced HPH mutants. Three of these mapped to *Pah*, the structural gene encoding phenylalanine hydroxylase [11]. Only 350 mice were needed to discover the second and third mutant mice. The second mutant, Pah^{enu2}, carries a missense mutation in codon 263 of the PAH polypeptide, severely reducing the activity of the enzyme. This mutant mouse, ENU2, made available through JAX, has been used in 170 publications, many of them recent (5 in 2021). Investigators studying gene therapy of heritable metabolic disease continue to use the ENU2 mouse strain through JAX as a test platform [12]. Dave and Alexandra fostered the connection with Savio Woo, the leader in this field of gene therapy originally connected to mouse model programs by Vernon Bode.

Success in approaching "saturation" in the development of embryonic lethal point mutations in the *T*-locus and mutations in the HPH metabolic system encouraged the laboratory to explore developing an extended set of ENU-induced mutations that modify the Min phenotype. In contrast to Bruce Beutler's success with "binary phenotypes" for natural resistance [10], with the help of Michael Newton this project addressed the serious statistical issues involved in characterizing quantitative phenotypes in the face of spontaneous variation [13]. The ambition to explore the entire network of genes impacting early stages in colon cancer has proven to be overly ambitious for the Dove lab. Harking back to the Latin theme of this essay, it is instructive to remember the poem, *Grandiose,* written by Patrick Dove around the age seen above in The Tuileries. His poem ends "Beware of grandiose!"

The 1993 article [11] reporting three mutant alleles in the *Pah* locus drew attention from the small community interested in developing a genetic analysis of neurobiological processes with mice. Larry Pinto at Northwestern, who had trained at Purdue with the neurogenetics pioneer Seymour Benzer, was in touch with neuroscience at Wisconsin through Donata Oertel. Larry became interested

in Alexandra's and Dave's HPH research. Importantly, Larry participated in the group of Joe Takahashi that, with Martha Vitaterna, developed ways to quantify the circadian rhythm of mice. The precision of this phenotyping combined with the efficiency of ENU mutagenesis to identify a mouse with an altered locomotory rhythm of running. A mouse presenting an altered activity rhythm, CLOCK, was identified in a cohort transferred by Dave McDonald from the mutagenesis program in McArdle. The "good fortune" of McArdle's mouse genetics — that had led to the discovery of the *T*^{Wis} allele, the Min strain, and the multiple Pah mutants — recurred in this collaboration. Instead of needing to screen 1500 mice to find a mutant in a single gene of interest, the CLOCK mutant mouse was number 27 in the cohort received from McArdle! A street philosopher once opined: "I believe in luck. The more I work, the more luck I get!"

Again, in biology we see the importance of a new mutant in establishing a field of study. Mice carrying the dominant *CLOCK* mutation have been made broadly available by JAX for research. To date, 1173 publications, 32 in 2021, cite its initial report [14]. The power of a new mutant strain in founding a field of study provides an independent example of the theme of this essay: *Finis Origine Pendet.*

In this spirit the contribution of the McArdle project has rested on several starting points. Alexandra brought to the effective use of the mouse her childhood enjoyment of this animal. Her facility in French was crucial to our ability to work with Jean-Louis and his French-speaking colleagues. Bill's familiarity with the different modes of carcinogen action came from his training in chemistry and immersion in the active McArdle faculty of the 1960s. Linda Clipson's longterm attention to the organization and presentation of results from large data sets has enabled the investigation of the genetics and biology of colon cancer in the university setting of McArdle. These inputs are "Homework" that bears fruit by "Doing Good Work."

In science, much benefit and pleasure can arise from finding ways to "just connect." Above we have seen ways in which Alexandra and others in the lab have supported the research of others by providing key methods and emergent strains. As François Jacob once said, "Without the right strain, you cannot do the right experiment!"

Our family has benefitted from its own versions of this principle. None of our three offspring, nor their spouses, has copied our professional paths. Each of the six pursues a unique creative professional path. But all are actively connected *inter se* and with us. A 2021 version of the image of The Tuileries at the beginning of this essay would have the next generation helping not only their own offspring to engage their world, but also us elders - even if using walkers! Any stumble is detected and shared among the three.

Just Connect

William (b.1966), Suzanne (b.1969) and Patrick (b.1967), July 2021

The fruits of science are usually unpredictable — if the unknown were known, it would not be unknown! Thus, new knowledge emerges from a darkness, presenting a new picture that is more than only "pretty." The scientist beholds a newly appreciated beauty.

Behold Beauty

Bill and Alexandra Dove, July 2021

Alexandra's family life is most important to her. Her life in science combines the pleasure of making a project work, joining that effort with colleagues, and communicating any fruits of that effort to other investigators who need "the right strain" for their own study. Unless she has contributed to the study hands-on effort to the design, data acquisition, or data analysis and presentation, Alexandra has declined gratuitous authorship. A community of scientists works best that way.

The personal and professional parts of Alexandra's life each encounter unplanned obstacles, arising at random. She emerges from these encounters with her own version of beauty, joining her family with the community of science.

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Acknowledgment: Linda Clipson has provided nimble and critical assistance in creating this essay. Patrick Dove has enhanced its clarity.

William Dove. December 17, 2021.