The DNA Provirus: Howard Temin's Scientific Legacy
Edited by G. M. Cooper, R. Greenberg Temin, and B. Sugden

Chapter 8

Mammalian Development and Human Cancer: from the Phage Group to the Genetics of Intestinal Cancer

William F. Dove

PROLOGUE

Together in this volume, we construct a mosaic. I shall bring into focus one stone of that mosaic: the abiding interest in the intersection of mammalian development and human cancer that I have shared with Howard Temin.

The experimental format of my own research is the laboratory mouse. It is no coincidence that Howard's first serious venture into biology was taken in company with the laboratory mouse. As a high school student, he had the good fortune to participate in a summer research program at C. C. Little's mouse mecca, The Jackson Laboratory in Maine. It is also no coincidence that Alexandra Shedlovsky, Amy Moser, and I, along with our McArdle Laboratory colleagues, continue to relate to the resources of The Jackson Laboratory in developing mouse models with which to study human health and disease. As many know from personal scientific experience, one must remain vigilant not to presume identity between mouse and human. In this essay, I discuss a mouse model for intestinal cancer, Min (for multiple intestinal neoplasia). We shall see homology to the human condition but not identity.

Howard's interest in development was fostered during his graduate career at the California Institute of Technology (Caltech) by a novel fusion to the emergent discipline of virology. When asked how he got into cancer research, Temin replied,

> As a graduate student I was interested in *cell development,* and the Rous sarcoma virus provided a system to change normal cells into other kinds of cells.
>
> —McArdle Laboratory, Nobel Prize press conference, October 1975 (emphasis added)

Howard's initial doctoral research home, the laboratory of embryologist Albert Tyler, did not permit the speed and quantitative character of experimental analysis

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that was being fostered at Caltech by the Phage Group, founded by Max Delbrück. Delbrück served on Howard's thesis committee in 1960, just before I, a doctoral student in physical chemistry at Caltech, began to work in the Phage Group. Work from my laboratory at Wisconsin on regulation in bacteriophage lambda (7) and on the cell cycle and cytoskeleton of *Physarum polycephalum* (2) has continued the tradition of the Phage Group. Now I am studying mammalian biology, and again, it is no coincidence that the research on development and neoplasia which I discuss here draws strongly from the tradition of the Phage Group. Howard and I have marveled at the connectivity of science that generates such apparent coincidences.

Let me finish this prologue by drawing your attention to ways in which Howard Temin continued to nourish his early interest in development while his career as a virologist expanded:

> In speculating about the origin of viruses with this special mode of information transfer, RNA → DNA, I felt that [this] information transfer *might play a role in normal organisms*.
> —H. M. Temin, Guest editorial: The Protovirus Hypothesis (20) (emphasis added)

In this intentionally speculative essay, Howard considered the possibility that reverse transcription generates somatic diversity for metazoan development. In subsequent decades, Howard remained interested in this possibility. The fascination has been fueled by the continuing discovery of retrotransposons all the way back to prokaryotic genomes and by the observation of apparent cDNA integrations in complex genomes. Today, the question continues to burn, one of Howard's "eternal flames": What biological function(s) drives the evolution of retrotransposition?

INTRODUCTION

To focus on my stone in our mosaic: What are the molecular and biological intersections of mammalian development and human cancer? Specific evidence on these intersections comes from the analysis of mutants, mouse or human. In the simplest case, one determines what neoplastic processes occur spontaneously in a mutant that has lost the function of a single developmental gene. In human cancer genetics, this case is approximated by families in which a mutant allele inherited in heterozygous form predisposes the individual to a particular cancer syndrome. Often, but not always, it is found that the neoplastic lineage has somatically lost the remaining normal allele that was present in the zygote.

Familial retinoblastoma is the paradigm: complete deletions of *RB* predispose heterozygotes to the disease, which is manifested by expansion of the population of a cell type that shows markers characteristic of early retinoblasts. The tumor lineage has somatically lost the remaining normal allele by one of several possible

mitotic mechanisms. A number of other cases have been found in the human: neurofibromatosis types I and II, Wilms' tumor, multiple endocrine neoplasia, familial adenomatous polyposis, and breast neoplasia (BRCA1 and BRCA2).

A full exploration of the intersection with normal mammalian development of these loss-of-function neoplasms requires analysis of embryos homozygous for the mutant allele. Generally speaking, material for this analysis is not available for the human. As the gene responsible for the human predisposition is identified, it has become possible in general to derive by gene targeting a mutant mouse line carrying a mutated allele of the mouse homolog. One intercrosses mice heterozygous for such a targeted allele and analyzes embryos that are homozygous for the mutant defect.

This exploration has been energetically pursued with mixed results. Mice homozygous for targeted loss-of-function alleles of the mouse homologs for *Rb* and *Nf-1* show defects in embryonic development. The defects are manifested after midgestation, affecting only certain organs.

However, the neoplastic phenotype of heterozygotes for targeted alleles of *Rb* and *Nf-1* does not encourage use of the mouse model for understanding the specific intersection of development and human cancer in which each of these genes is involved. The tissues that are predisposed to neoplasia differ in the mouse and the human. On this score, perhaps it will be informative to study development in mouse embryos homozygous for the *Min* mutant allele of the *Apc* gene. Here, the heterozygous mouse line shows a neoplastic phenotype that closely resembles that of corresponding human familial adenomatous polyposis families. This neoplastic process can best be appreciated in the context of the biology of the intestinal epithelium of the mammal. The intestinal epithelium is a self-renewing tissue, even in the adult mammal. Proliferation is limited to crypts. As cells exit the crypt and terminally differentiate, they move up the fingerlike villi that line the small intestine. These villi are replaced by a cuff that surrounds the crypt of the large intestine. The total mitotic risk of the intestinal epithelium is enormous. Clearly, this risk is attenuated by the strategy of shedding one daughter lineage from each stem cell mitosis within the crypt. But whether and how the retained daughter lineage reduces its risk of mitotic error remain deep mysteries (3).

The Min mouse carries a germ line mutation in one allele of the *Apc* gene and develops scores of adenomas in both large and small intestines during its lifetime. Homozygotes for the *Min* mutation cannot be found among the live-born progeny of a cross between two *Min*/+ heterozygotes (12). Thus, the *Apc* gene is an essential developmental gene in the mouse with an appropriate heterozygous phenotype of intestinal neoplasia. Indeed, the essential starting point of the experimental strategy used in our laboratory is finding the appropriate mutant phenotype at the level of the intact organism. The development of efficient point mutagenesis of the mouse germ line with ethylnitrosourea has made this strategy feasible (5, 15–17, 22). Among the more than 14 recessive mutations in essential developmental genes that we have isolated and mapped, only *Min* shows a pronounced heterozygous neoplastic phenotype (15a). I focus in this essay on two genes: *Apc* on chromosome 18, in which the *Min* nonsense allele has been induced by ethylni-

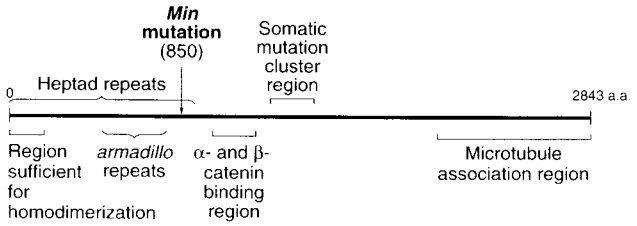

FIGURE 1. The APC/Apc polypeptide. a.a., amino acids.

trosourea (12, 18), and the first modifier-of-Min locus, *Mom-1*, on chromosome 4 (4).

The *APC/Apc* gene of mammals encodes a giant 300-kDa cytoplasmic polypeptide of unknown function (Fig. 1). Several regions involved in macromolecular interactions have been identified for *APC/Apc:* a homodimerization domain near the amino terminus; heptad repeats characteristic of polypeptides that can form coiled-coil oligomers throughout the amino-terminal third of the molecule; seven 42-amino-acid repeats homologous to those found in the plakoglobin homolog of *Drosophila melanogaster* (in *armadillo*) and in the *smg* family of 21-kDa G proteins of mammals (14); and a region that binds α- and β-catenin, polypeptides that also bind the calcium-dependent cell adhesion molecule E-cadherin (8). In the carboxy-terminal region of the *APC/Apc* polypeptide is a region that associates with the microtubular cytoskeletons of mammalian cells.

Using the Min mouse, I address five questions. (i) What is the developmental potential of the *Min/Min* zygote? (ii) What somatic genetic events occur when tumors form in *Min/+* heterozygotes? (iii) What is the developmental potential of *Min/0* adenomas? (iv) What other loci in the mouse genome can modify the severity of *Min*-induced neoplasia? (v) For each gene that is implicated in the developmental or neoplastic action of *Apc*, how is gene action partitioned between the tumor lineage, the microenvironment, and the systemic environment of the host?

DEVELOPMENT OF THE *Min/Min* EMBRYO (13)

When mice heterozygous for the *Min* nonsense allele of *Apc* are intercrossed, no live-born *Min/Min* homozygous progeny are found (12). Abnormal embryos can be found in intercross litters as early as 5.5 days of gestation, soon after implantation. Control crosses show abnormal embryos at much lower frequencies. It is, however, one thing to report abnormal embryos in the experimental crosses but quite another to demonstrate that the abnormal early embryos are the mutant homozygotes. We have therefore invested the effort needed to determine the genotypes of the individual pregastrulation embryos from these matings.

The most powerful method for determining genotypes of the mutant and wild-type sites of the *Apc* gene involves using mismatched PCR primers that amplify the region of the gene surrounding the *Min* site and create an allele-specific *Hin*dIII site in the wild-type product and control *Hin*dIII sites in both the *Min* and the wild-type products (9). Using this method, we have determined the genotypes of embryos at 7.5 days of gestation that have been dissected away from much of the maternal decidual tissue. The mutant homozygotes are poorly developed at this stage.

What tissues are specifically affected in the *Min/Min* embryo? Embryos isolated within their decidual swellings and stained by hematoxylin and eosin include abnormal embryos that are severely affected in their primitive ectoderm at the distal end of the egg cylinder at 6.5 days of gestation, before the onset of gastrulation is heralded by the formation of the primitive streak. Embryos of this stage have been dissected away from the decidua, prepared for histological analysis, and scored morphologically, and finally, their genotypes have been determined from DNA isolated from histological sections.

Determining the genotypes of embryos that have been sectioned for histology requires a demanding protocol of DNA isolation from single sections and the use of informative PCR markers. The presence of hematoxylin and eosin compromises genotyping by the site-specific protocol discussed above. The markers of choice, therefore, have been polymorphic, simple-sequence repeat markers that closely flank the *Apc* locus. The *Min/Min* embryos have been generated by intercrossing (AKR × C57BL/6-*Min*/+)F_1 parents. Embryos shown to be homozygous for the wild-type region surrounding *Apc* show normal primitive ectoderm. In contrast, embryos homozygously mutant for the *Apc* region show the same typically abnormal phenotype seen in the embryos that were fixed within the decidua, i.e., underdeveloped primitive ectoderm but relatively normal extraembryonic ectoderm and endoderm.

We have investigated earlier stages of development, i.e., those immediately following implantation. All 38 embryos from heterozygote intercrosses developed primitive ectoderm, though a subset, perhaps the mutant homozygotes, seemed to be retarded. This stage does not yet permit explicit genotyping by which we can rigorously assign the genotype, but we can conclude that the *Min/Min* embryo forms primitive ectoderm but is blocked in the maintenance or expansion of this totipotent early embryonic tissue.

What cell types can develop in the absence of the normal *Apc* product? Looking late in development, we find that the invasive trophoblast giant-cell population is abundant and apparently normal, even at 10.5 days of gestation.

In summary, an embryo homozygous for the *Min* allele of *Apc* is affected in the development or persistence of the primitive ectoderm soon after implantation. The primitive ectoderm has been shown to be the embryonic precursor of all three germ layers of the mouse embryo. However, the mutant condition is not one of general cell lethality; the invasive trophoblast giant cells, at least, can persist in the absence of wild-type *Apc*. One rule that we derive from this study is that the developmental genes that predispose to neoplastic processes by loss of function must be fundamentally regulatory rather than necessary for cell viability.

SOMATIC GENETIC EVENTS DURING TUMORIGENESIS IN *Min*/+ (9)

If the *Apc* gene controls neoplasia by a simple loss-of-function process, one would expect tumors arising in *Min*/+ carriers to lose all activity of the normal *Apc* gene. In the corresponding case in the human, however, at most only 70% of tumors show detectable loss of the normal allele.

Loss of the wild-type *Apc* site has been analyzed by sectioning tumors, staining 1 of every 10 sections, locating the region of neoplastic growth, and isolating DNA from the unstained slides. The site-specific PCR assay described above was used to quantitate the ratio of wild-type to *Min* mutant DNA in the tumor (Fig. 2). For every one of 47 adenomas, extensive loss of the normal allele was observed in tumor samples but not in adjacent normal tissue. Thus, even for adenomas smaller than 1 mm in diameter, most cells in the tumor have lost the function of *Apc*.

These experiments, performed on an inbred background, have been extended to an (AKR × B6)F_1 background so that the fate of the entire wild-type chromosome can be followed (Fig. 3). At loci everywhere along chromosome 18, the allele linked to the wild-type *Apc* gene is lost in the 50 tumors analyzed. Control loci on other chromosomes have not shown such losses in *Min*-induced intestinal tumors.

Titration of the copy number shows that the remaining B6-*Min* chromosome is haploid, not reduplicated. Therefore, in the Min mouse, it seems that all adenomas are hemizygous for the mutated copy of chromosome 18. However, although all tumors show extensive loss, each tumor retains 10 to 20% of cells with the wild-type allele. Although great care was taken to analyze only the neoplastic region of the tumor, it is conceivable that DNA from normal cells contaminated the samples. Alternatively, it is conceivable that the adenoma is not clonal.

Three scenarios for understanding the somatic events underlying adenoma formation in the mouse are now under consideration (Fig. 4). The first assumes that the *Min* nonsense allele has simply lost *Apc* function and that adenoma formation requires only a mitotic error in which the wild-type homolog is lost. A second suggests that the nonsense allele of *Min* has a heterozygous cellular phenotype, for instance, the enhancement of mitotic errors to produce the hemizygous adenoma. Regarding this second scenario, it is interesting to recall that the APC polypeptide

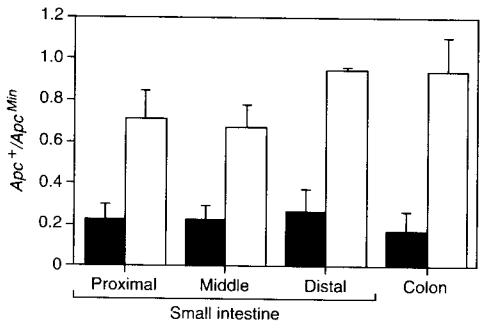

FIGURE 2. *Apc* allelic ratios for intestinal samples from B6-*Min* mice. ■, Tumor; □, control.

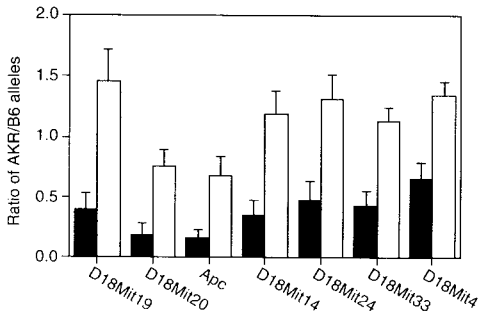

FIGURE 3. Allelic ratios for intestinal regions in (AKR × B6-*Min*)F_1 Apc^{Min}/Apc^+ mice. ■, Tumor; □, control.

interacts with the microtubular cytoskeleton and so perhaps interacts with the mitotic apparatus as well. A third plausible scenario underlines the fact that we do not yet know that total loss of Apc function is sufficient for adenoma formation, even if it is necessary. There may be yet other somatic mutations, symbolized by *M*, that are also necessary. At least three genetic events are necessary for adenoma formation in this case.

THE DEVELOPMENTAL POTENTIAL OF *Min*/0 TUMORS (10)

In sections of *Min*-induced tumors, we have detected by immunohistochemistry the expression of cellular markers for three differentiated cell types of the intestinal epithelium: the Paneth cell (lysozyme), the enteroendocrine cell (serotonin), and the enterocyte (fatty acid-binding protein). Even in invasive tumors in older Min animals, we continue to find these markers.

It seems that the loss of Apc function does not compromise the expression of these differentiation markers of the intestinal epithelium, but we must remain cautious on at least two counts. First, we do not yet know whether the differentiation marker is expressed by the cells that have lost the Apc function rather than by the minority of cells in the tumor that may still retain it. Second, one differentiation marker does not constitute full demonstration of a cell type.

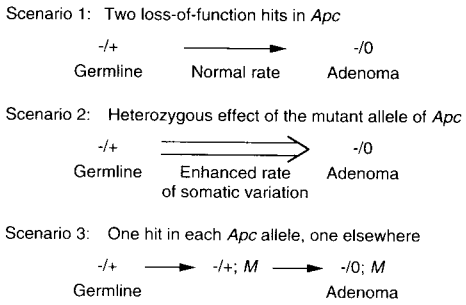

FIGURE 4. Clonal evolution of the adenoma.

WHAT OTHER LOCI INTERACT WITH Apc^{Min} IN TUMORIGENESIS?

Genetic changes accumulate as cancer progresses. Indeed, we are becoming acutely aware of situations in which cancer is driven by genetic instability syndromes.

If both *Apc* and *p53* are mutated in the germ line, is the probability of tumor formation enhanced? Note that the *Min*-induced adenoma has lost a copy of chromosome 18, and *p53* lesions are commonly associated with aneuploidy. We have intercrossed heterozygotes for a targeted loss-of-function allele of *p53* in the presence of the *Min* mutation and determined the multiplicity, size distribution, and histology of the resultant tumors. No significant differences have been detected for any of these three parameters at 80 days in the development of intestinal tumors (6a).

We have carried out an explicit search for loci that do modify the action of the *Min* mutation. Min mice on the sensitive C57BL/6 genetic background have been crossed with other inbred mouse strains, and the tumor multiplicity of the F_1 hybrids has been determined (Fig. 5). On the sensitive B6 background, shown as white bars in the histogram in Fig. 5, the mean tumor multiplicity is 29. In contrast, for Min animals in the (AKR × B6)F_1 generation (black bars, Fig. 5), the mean tumor multiplicity is only 5. The genetic basis of this reduction in tumor multiplicity has been determined by analyzing a population of animals in which the F_1 generation has been backcrossed to the sensitive B6 parent (gray bars, Fig. 5). Animals with low tumor multiplicity tend to inherit the AKR allele at a locus on mouse chromosome 4 that we call modifier-of-Min 1, or *Mom-1*. Those with a high tumor multiplicity tend to be homozygous for B6 alleles in the region of *Mom-1*. The location of *Mom-1* has been estimated to a resolution of about 15 centimorgans (cM).

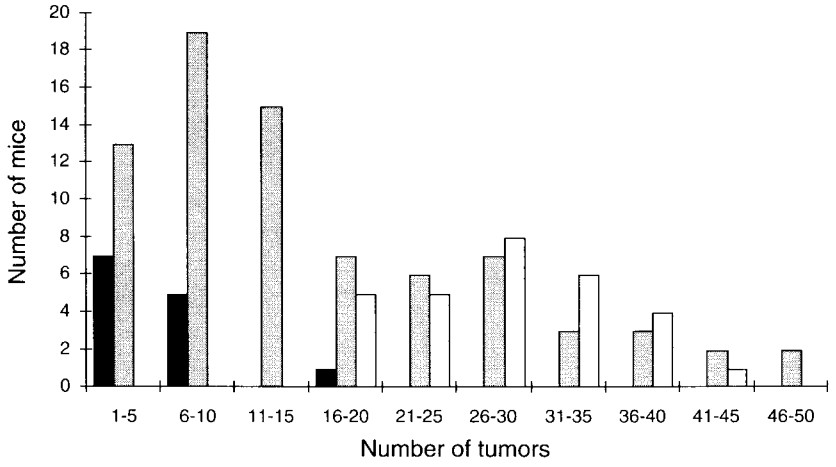

FIGURE 5. Tumor multiplicity in *Min*/+ mice from the AKR backcross. ■, AKR × B6 mice; ▨, B6 × (AKR × B6) mice; □, B6 mice.

Mom-1 accounts for only a portion of the dominant effects of the AKR background on *Min*-induced intestinal neoplasia. However, no other dominant resistance allele can be mapped from these backcross data. Even further suppression of tumor multiplicity can be seen when the AKR background is homozygous (9a).

The effects of the AKR allele of *Mom-1* have been detected in heterozygotes; they are at least partially dominant. With congenic *Mom-1* derivatives, we are actively investigating whether two copies of the resistant AKR allele confer additional suppression of *Min*-induced neoplasia. Further, we want to know whether the *Mom-1* locus affects the other neoplastic processes to which Apc^{Min} predisposes an organism.

What molecule is encoded by the *Mom-1* locus? A region as large as 15 cM has many interesting candidates (4). To move toward a more restricted set of candidates, a resolution for the map position of *Mom-1* in the range of 1 to 2 cM is now being achieved by the analysis of recombinant chromosomes that dissect this region (7a).

The *APC/Apc* gene seems to be broadly expressed, as judged by RNA analysis, yet its mutations are normally first detected by their effects on the intestinal epithelium. This apparent tissue specificity might reflect an enhanced risk of somatic mutations or mitotic errors in the intestinal epithelium, with its very high proliferative index. However, further studies of human kindreds affected in *APC* and of Min mice have shown that other tissues are predisposed to developmental malformation or neoplasia by mutations in *APC/Apc* (Table 1).

One neoplastic pathway found in Min mice but not yet associated with germ line *APC* mutations in humans is that of the mammary gland (11). The penetrance of this phenotype is normally 10% or less in Min mice. A similarly low penetrance in the human might have escaped detection owing to a high background of other causes. Indeed, Thompson and his colleagues (21) have reported somatic mutations in *APC* in human mammary tumors.

Overall, we believe that the Min strain permits a general scan of the mammalian gene pool for genes that can modify the risk of particular neoplasms. Over the past decade, we all have been impressed by the steady progress in the identification of disease-predisposing genes in the human population, from familial hyper-

TABLE 1
Heterozygous phenotypes associated with germ line mutations in *APC/Apc*

Human	Mouse
Multiple colon adenomas	Multiple colon adenomas
Small intestine adenomas	Small intestine adenomas
Desmoid tumors	Desmoid tumors
Epidermoid cysts	Epidermoid cysts
Gastric polyps	Mammary adenocarcinomas
Osteomas of skull and mandible	Mammary keratoacanthomas
Hypertrophy of retinal pigment epithelium	
Abnormal dentition	

cholesterolemia to retinoblastoma to cystic fibrosis, but most of these genetic predispositions show variable expressivity ranging all the way to incomplete penetrance. Perhaps a part of this variation is due to modifier alleles at unlinked loci in the human. However, quantitative modifiers of disease severity are difficult to map within the available human disease families. By contrast, loci such as *Mom-1* can be mapped in appropriate mouse crosses. Thus, my colleagues and I foresee decades in which mouse strains that closely simulate human disease predispositions will be used to detect protective genes (6). Candidate genes detected by mouse genetics can then be tested within the corresponding human kindreds to determine whether corresponding resistant alleles exist in the human. Even if such mutant alleles cannot be found in the human population, the molecular identification of loci such as *Mom-1* by using the molecular genetics of the mouse may present opportunities for intervention in human disease.

It will be interesting to see what comes of this.

GENE ACTION INTRINSIC OR EXTRINSIC TO THE TUMOR LINEAGE

To carry forward the analysis, we need a way to distinguish between genes whose action is intrinsic or autonomous to the tumor lineage and those that act from surrounding tissue. Indeed, for several years in the 1960s, Howard Temin was involved in studying both the intrinsic and the extrinsic sides of cell conversion by Rous sarcoma virus (19). In this system, the transforming provirus is an element intrinsic to the neoplasm, and insulin-like growth factors to which converted cells respond differentially are extrinsic. The properties of the tumor can in principle be altered by changes either in the provirus or in the source of any differential growth factors.

We have recently made an observation consistent with the hypothesis that *Mom-1* may act extrinsically to the tumor lineage in *Min*-induced adenomas (8a). When tumors form in (AKR $\times$ B6-*Min*/+)F_1 hosts, the region surrounding the *Mom-1* locus remains heterozygous in the tumor lineage. Unlike the *Apc* locus, where the protective wild-type allele is always lost, the protective allele of *Mom-1* seems to be retained. One interpretation of this result is that *Mom-1* controls tumor formation from surrounding tissue.

This suggestion is far from proven. We want to be able to label one of the genetic components of the chimera in such a way that cells derived from it can be recognized unambiguously no matter what the state of differentiation or neoplasia. We are seeking a histochemical cell lineage marker to permit such an analysis of *Mom-1* action. Over the coming years, we may be able in these ways to tease apart the networks of genes that impinge upon the pathways of neoplasia predisposed by mutation of the developmental gene, *Apc*.

EPILOGUE

Howard and I talked more than once about the special opportunity for doing science away from the mainstream bustle of the urban centers of the United States. The metaphor he used was Backwater Science.

It is in the shadows that momentous encounters occur.

—Alfred Fabre-Luce

Every one of us in research knows the brave solitude of striking out into unmapped territory. Howard and I have enjoyed the special opportunity to do this in the Wisconsin community. In particular, McArdle Laboratory has been built by solid citizens of the cancer research community: H. P. Rusch, V. R. Potter, J. A. Miller, R. K. Boutwell, E. C. Miller, C. Heidelberger, and G. C. Mueller, the founders who hired Henry Pitot, Howard Temin, and Waclaw Szybalski in 1960 and Charles Kasper and me a few years later. These are the early investigators responsible for the well-built cabin and research discipline from which Backwater Science could be pursued.

In commemoration of Howard, we marvel at the series of successes in his research and that of his protegées. Let me add some texture from the early days of the mid-1960s. From my very first week in Madison in 1965, five of us gathered for Monday lunch. I thought of this lunch gathering as The Caltech Group. (In fact, only Howard, Millard Susman, and I were direct emigrés from Caltech; Masayasu Nomura and Bernie Weisblum were indirect descendants of the Caltech Phage Group through their experiences with Seymour Benzer at Purdue.)

In 1965, the notion of retrotranscription was far from accepted, much less the notion of retrotransposition. There were other interpretations of the effects of inhibition of DNA synthesis on the life cycle of Rous sarcoma virus, and Howard's hybridization evidence for a DNA provirus, offered a decade in advance of Southern blotting, was necessarily marginal.

> Hybridization experiments have been carried out, and there is an increase in . . . the DNA from infected cells, as compared with DNA from uninfected cells (Temin, 1964b). *These results, however, are as yet unconfirmed.*
>
> —H. M. Temin, Studies on Carcinogenesis by Avian Sarcoma Viruses. IV. The Molecular Biology of Viruses (1967) (emphasis added)

During those days, stretching into years, we lunching colleagues related to the DNA provirus hypothesis not as established fact, not as a loyalty contest between believers and nonbelievers, but as a plausible idea looking for stronger tests of its viability. The bromodeoxyuridine sensitization experiments of David Boettiger (1) evolved from Monday discussions of The Caltech Group. It is in the highest sense of Karl Popper that Howard would refer to his own claims as "unconfirmed." More important in science than to be right is to do it right.

There are many visionaries. The visions that move successfully into our base of knowledge are the ones that come into direct contact with feasible experiments. Howard's DNA provirus vision, based on strong biological inference, came into contact first with the experimental recognition of virus-borne enzymes by Kates and MacAusland, among others. Later, the vision contacted vast improvements in DNA analysis with restriction enzymes, mammalian cell DNA transformation, and low-noise Southern blotting.

All of these were matters of science, carried on quietly both in the backwaters and the mainstream over the 1960s. But science also has an essential public face, which can be ugly.

> Dr. H. M. Temin's *tantalizingly incomplete* work . . . suggests that avian sarcoma virions contain an enzyme capable of making DNA using a single stranded RNA template. . . . Temin *claims* that avian sarcoma virions, after treatment *with an unspecified detergent,* will incorporate deoxynucleoside triphosphates . . .
>
> —*Nature* (London) **226**:1003 (June 13, 1970) (emphasis added)

> The discovery of this unprecedented enzyme . . . is an *extraordinary personal vindication* for Dr. Howard Temin.
>
> —*Nature* (London) **226**:1198 (June 27, 1970) (emphasis added)

In a span of 2 weeks, one could read descriptions of Howard Temin's research that ranged from anonymous condescension to public idolatry. I have always believed that Howard had a sufficient store of skepticism to survive any such waves of public notoriety and fame. He recognized clearly the competitive urge that drives so much creative effort, both scientific and journalistic.

The vital partner to competition is a sense of community. When Howard was awarded the Nobel Prize along with Renato Dulbecco and David Baltimore, he gave a prominent nod of gratitude to the virology community. In recent years, Howard invested significant personal energy as a de facto ombudsman for certain ethical disputes within that community.

Here in the backwaters of Wisconsin, especially during Howard's brave confrontation with cancer, his colleagues have been paramount, with his family at the center.

I have given here a strictly personal view of current issues in the biology of cancer and in the outlook of Howard Temin. There should be other views. For example, had Sewall Wright lived to 105, as expected, right now he would be arguing with Howard that retrotranscription and retrotransposition have been selected not for a role in development but for their role in the genetic variation of populations.

ACKNOWLEDGMENTS. Studies of the Min strain, following its discovery by Amy Moser in my laboratory, have grown to involve many constructive collaborations. Biological and genetic studies of Min at McArdle Laboratory have involved Amy Moser; doctoral students Cindy Luongo, Karen Gould, and Al Shoemaker; our specialists, Melanie McNeley, Darren Katzung, Linda Clipson, and Natalie Borenstein; and our consultants, Norman Drinkwater and Henry Pitot. Our studies of general embryology and neoplasia in the mouse have depended on Camille Connelly, Paraj Mandrekar, and Alexandra Shedlovsky. To study mammary neoplasia, we have worked with Jill Haag and Michael Gould of the Clinical Cancer Center at Wisconsin. The intestinal cell type analysis was carried out with Kevin Roth and Jeff Gordon of Washington University, St. Louis. The molecular genetics of *Apc* has benefited from close collaboration with Li-Kuo Su, Dan Levy, Stan Hamilton, Ken Kinzler, and Bert Vogelstein of Johns Hopkins University. The knockout genetics of *p53* has involved Larry Donehower of Baylor University. To map the *Mom-1* locus, we have joined with Bill Dietrich and Eric Lander of The Whitehead Institute, Massachusetts Institute of Technology. Finally, the analysis of *Min/Min* embryos has enjoyed the expertise of Richard Gardner of the Imperial Cancer Research Fund, Oxford.

Studies of the Min strain will grow over the coming years now that it is genetically inbred and provided by The Jackson Laboratory to all investigators worldwide.

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