Perspectives

Anecdotal, Historical and Critical Commentaries on Genetics

Edited by James F. Crow and William F. Dove

Gisela Mosig

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GISELA Mosig enjoyed a distinguished career in molecular genetics during a life richer in adventures and disasters than is common among scholars. The formal outline of her career conceals much. She was born in Saxony on November 29, 1930. She left East Germany as a teenager, did her undergraduate studies at the University of Bonn, and went on to obtain a doctoral degree in plant genetics at the University of Cologne in 1959. She worked for 3 years as a postdoctoral fellow with A. H. Doermann at Vanderbilt University in Nashville, Tennessee, and continued for another 3 years with A. D. Hershey at the Carnegie Institution of Washington in Cold Spring Harbor, New York. In these two intervals she became deeply immersed in the general and molecular genetics of the T-even phages. She joined the Department of Molecular Biology at Vanderbilt in 1965 (becoming a citizen in 1968) and worked there until her death on January 12, 2003, leaving behind an influential corpus of discoveries in the molecular biology of phage T4 (Figure 1).

Beginnings: During the war, which ended when she was 14, Gisela grew up on a farm and developed interests in biology and genetics. In high school, her instruction in Mendelian genetics changed abruptly to indoctrination in the fraudulent theories of Trofim Lysenko. From the very start of her schooling, Gisela was convinced of the importance of logic and evidence to theories that related to biology and the world in general. Her knowledge of Darwin's work and the theory of evolution could not be reconciled with a version of science based on political expediency. Ultimately, Gisela was to interpret much of her work within the framework of evolutionary theory. With this mind set, she found the climate of communist East Germany intolerable and, at the age of 18, managed to flee to West Germany. On the pretense of going into the mountains for a picnic, she and a friend escaped East Germany by riding their bikes at a breakneck downhill pace out of the mountains, avoiding both patrols and mine fields. All she took with her was what she could carry on the bike. Nonetheless, she was ecstatic to be free. As they crossed the border they came across some wild strawberries, and she recounted that nothing else ever tasted as good as those berries.

Gisela did her undergraduate studies at the University of Bonn. She continued with her focus on biology and genetics at Cologne, obtaining her Doktor Rerum Naturalis in 1959 based on studies of petunia hybrids (Mosig 1960a,b). While at Cologne, she met A. H. (Gus) Doermann, a visiting Vanderbilt professor and early leader in the genetics of phage T4, who recruited her to Vanderbilt as a postdoctoral fellow. Gisela's first T4 article (Mosig 1962) showed that recombination frequencies increase with increasing multiplicities of infection, a result that she recalled with affection in a conversation shortly before her death. This was one of the few uncomplicated articles that she was to write in a lifetime of intense research.

Entry into T4 research: Using technically challenging single-burst experiments, Gisela began to investigate the properties of "petite" T4 particles containing shortened genomes (Mosig 1963a,b, 1970a,b; Mosig and Ehring 1968; Mosig *et al.* 1968, 1971, 1972b; Mosig and Werner 1969; Marsh *et al.* 1971; Walker *et al.* 1972). Although these reduced particles were not infectious singly, multiple infection often produced viable progeny, and the ends of petite genomes exhibited gradients of recombination potential. Gisela's work and the contem-

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FIGURE 1.—Gisela in her Vanderbilt laboratory. This photograph, probably taken in the middle to late 1970s, was found in her files without attribution.

poraneous work by the Eugene group led by George Streisinger and Frank Stahl (STREISINGER *et al.* 1964; SÉCHAUD *et al.* 1965) supported the proposals that T4 has a circularly permuted, terminally redundant chromosome and two very different kinds of recombinationally generated heterozygotes. This chain of articles firmly established Gisela as an inventive and prolific investigator and revealed her ability to conduct and describe exceedingly detailed experiments and to draw from them fine and carefully qualified conclusions. Her style was already reminiscent of that of Barbara McClintock and, as with McClintock's articles, it was often necessary to read Gisela's articles repeatedly and minutely, first to understand them and then to think about them critically. For her personal perspective on this confirmation into the "phage church," see MOSIG (2000).

The T4 replication-recombination connection: The essential role of recombination in successful DNA replication is now recognized in both prokaryotic and eukaryotic systems. As a result of her pioneering studies, Gisela was among the first to appreciate the importance of recombination intermediates in establishing new replication forks. Her work extended from the early studies of short genomes, suggesting preferential replication of termini, through electron microscopic analyses of replication intermediates and characterizations of the genetic requirements for early and late T4 replication, to her recent characterization of origin-initiated replication at T4 *oriE*.

Classic studies of conditionally lethal T4 mutants (EPSTEIN *et al.* 1963) identified a phenotype called DNA arrest that began replication normally but stopped abruptly. Most of these mutants turned out to carry mutations in genes encoding recombination proteins. Characterization of these mutants led several investigators to invoke two separate modes of T4 DNA replication, origin initiated at early times and recombination dependent at late stages (BROKER 1973; BROKER and DOERMANN 1975; KOSTURKO and KOZINSKI 1976; HUTCHINSON *et al.* 1979). Gisela agreed that T4 used both modes but argued that the two were extensively interconnected throughout the infection cycle (MOSIG 1987, 1998; MOSIG *et al.* 2001). She and Richard Dannenberg showed that when recombination was prevented, most early replicating molecules initiated from a single origin. After multiple infection, recombinational intermediates appeared before the end of the first round of replication, and many of these molecules were highly branched. Thus, recombination could begin as soon as origin-initiated replication reached an end of a molecule (DANNENBERG and MOSIG 1981, 1983). Tom Broker's analysis of recombination intermediates after multiple infection with mutants unable to synthesize DNA established that recombination could also occur in the absence of replication (BROKER and LEHMAN 1971; BROKER 1973). Gisela showed that recombination increased greatly with concomitant origin-initiated replication, presumably because replication increased the supply of 3′ ends for strand invasion and of gaps between Okazaki fragments for single-strand annealing (LUDER and MOSIG 1982).

The T4 transcription-replication connection: It still remained unclear why origin-dependent replication was confined to early times and whether the RNA primer, which was presumably used to initiate DNA synthesis at T4 origins, was made by RNA polymerase or by a primase. T4 uses the *E. coli* RNA polymerase for all of its transcription but modifies it at different stages of the infection cycle (STITT and HINTON 1994; WILLIAMS *et al.* 1994; Figure 2). Early genes are transcribed by unmodified RNA polymerase and middle genes by the same polymerase modified by the T4 MotA and AsiA proteins, while the transcription of late genes requires two new phage-encoded factors, transcription activator gp33 (gene product of gene *33*) and σ factor gp55, which replaces the host σ^{70}. Rifampicin inhibits RNA synthesis at each of these stages. Gisela favored the idea that origin synthesis is primed by RNA polymerase because mutations in the T4 primase gene have a DNA-delay phenotype (EPSTEIN *et al.* 1963), indicating that priming by T4 primase is not essential at origins. Moreover, she had shown that T4 replication occurred normally in host *dnaG* primase mutants (MOSIG *et al.* 1972a; BRESCHKIN and MOSIG 1977). She suggested that T4 origins were primed by the form of host RNA polymerase used for early or middle transcription and that this function was inhibited when the polymerase was later modified by gp33 and gp55. LUDER and MOSIG (1982) tested this hypothesis by assessing the effect of rifampicin on replication by a quadruple *33*$^-$ *55*$^-$ *46*$^-$ *47*$^-$ mutant defective in both late transcription and recombi-

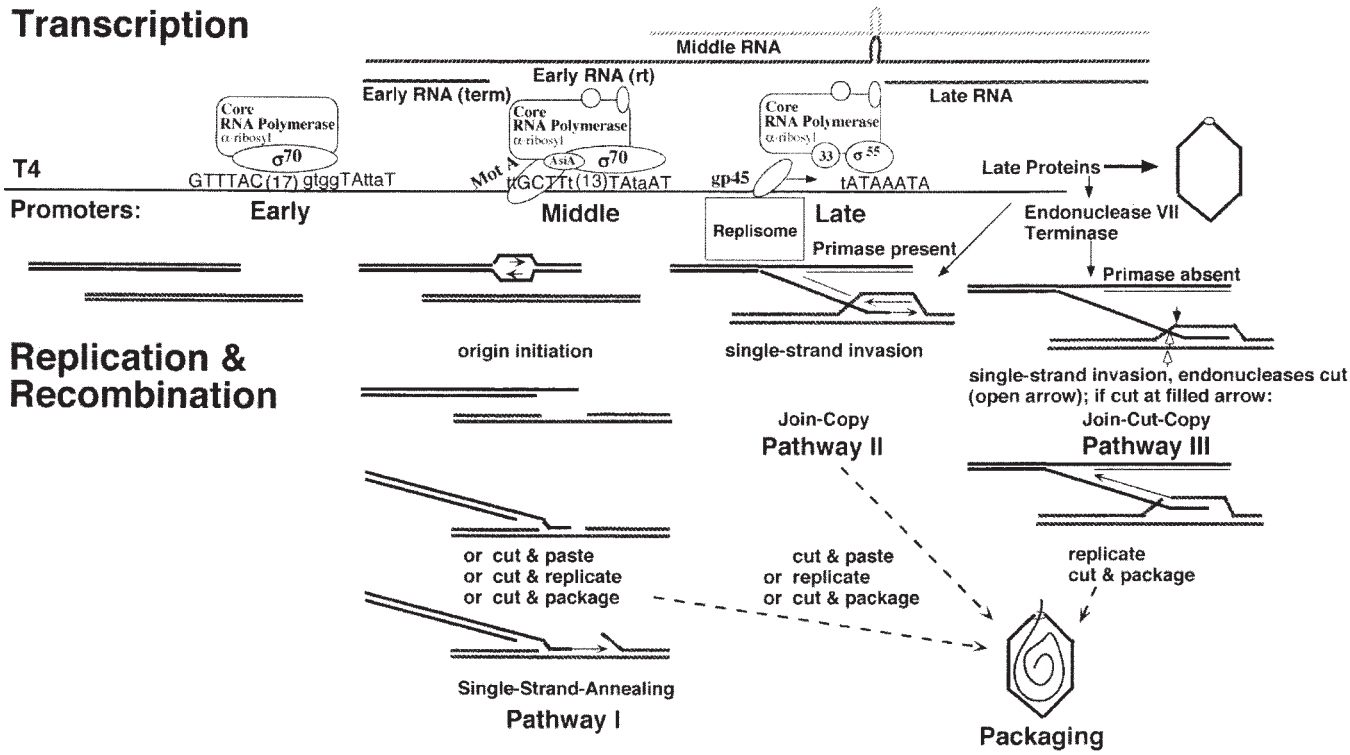

Figure 2.—Gisela's diagram of the relationship between T4 transcription and the different mechanisms of DNA replication and recombination. She used ever-evolving and ever more complex versions of this table for many years. *E. coli* RNA polymerase is sequentially modified for transcription from T4 middle and late promoters. Middle transcription is needed to prime leading-strand synthesis on many T4 origins and to transcribe genes encoding T4 replication proteins. Late transcription is needed to make endonuclease VII and terminase. Light lines represent strands of homologous regions of DNA and the shortest arrows point to positions of endonuclease cuts. Reprinted with permission from Miller *et al.* (2003).

nation. (Genes *46* and *47* encode essential recombination proteins.) Their conclusion that T4 origin synthesis begins with transcription from early or middle T4 promoters has been confirmed in studies of four of these origins (see below).

Gisela's view of the multiple connections among transcription, replication, and recombination is shown in Figure 2. She believed that recombination-dependent replication was essential for T4 for two reasons: it solved the problem of how to fully replicate the ends of the linear genome, and it allowed replication to continue after the host RNA polymerase was modified so that it could no longer transcribe from the early and middle promoters in the origins. Her finding that rifampicin-sensitive replication continued at late times in the quadruple mutant is consistent with the notion that modification of RNA polymerase by gp33 and gp55 is normally responsible for the inhibition of origin-dependent synthesis (Luder and Mosig 1982). The additional or alternative explanation that these modifications are required to transcribe a gene encoding a protein that inhibits origin-dependent replication was confirmed by recent studies showing that the late T4 protein UvsW is a helicase whose inappropriate early synthesis inhibits origin-dependent replication (Carles-Kinch *et al.* 1997).

T4 gp61 primase is required to initiate lagging-strand fragments *in vitro*, but the phenotype of a gene *61* mutant is DNA delay rather than the expected lack of any DNA synthesis. Gisela's genetic studies showed that the connection between recombination and replication resolves this paradox. In the absence of primase, the lagging-strand template remains single stranded and its 3′ end can invade a homologous molecule (the join-cut-copy pathway at the right side of Figure 2). Either endonuclease VII or terminase, both late proteins, could cut the complementary strand at the invasion junction, creating a 3′ end that could be extended by copying the lagging-strand template (Mosig *et al.* 1991; Mosig 1998).

Initiation from T4 replication origins: The search for T4 origins of replication was difficult and controversial because there turned out to be multiple origins whose relative use appears to depend on growth conditions (reviewed in Kreuzer and Morrical 1994). Four of these origins (*oriA*, *oriE*, *oriF*, and *oriG*) are now well characterized. Gisela played an important role in the early search for origins, using conditions that favored *oriA* and *oriE* (reviewed in Mosig 1983). She and her colleagues mapped transcripts and potential RNA-DNA transition points in these origins and in *oriF* (Mosig and Colowick 1995; Mosig *et al.* 1995; Vaiskunaite *et al.* 1999). In contrast to *oriA*, *oriF*, and *oriG*, which contain middle-

mode transcription promoters, they showed that *oriE* is transcribed from early promoters by unmodified host RNA polymerase (Vaiskunaite *et al.* 1999). They also identified the first origin-specific T4 proteins, RepEA and RepEB, and showed that binding of RepEB to one or more of a set of repeated sequences (iterons) within *oriE* was required for *oriE* function (unpublished experiments cited in Miller *et al.* 2003). At the time of her death, Gisela was testing her proposal that RepEB helped to load the T4 gp41 helicase at *oriE*.

Lessons from the complexities of T4 replication and recombination: In her talks and reviews (Mosig 1983, 1987, 1998; Mosig *et al.* 2001), Gisela emphasized repeatedly that while multiple overlapping and apparently redundant interconnected pathways for replication and recombination might be confusing to investigators, they were in fact beneficial to the organism. In her view, "different pathways are preferred under certain conditions, or with certain model substrates, and the elimination of one pathway channels intermediates into another" (Mosig *et al.* 2001, p. 8306). Multiple homologous recombination pathways have been delineated in *Escherichia coli*, but only recently has it been appreciated that the major role of these recombination pathways is the nonmutagenic repair of stalled replication forks (reviewed in Cox *et al.* 2000). Most of the T4 proteins required to create replication forks on recombination intermediates have functional analogs in *E. coli* and eukaryotes. Gisela was understandably pleased that so many speakers at a recent National Academy of Sciences colloquium on "Links Between Recombination and Replication: Vital Roles of Recombination" acknowledged their debt to the early studies of recombination-dependent replication in T4.

Complex connections: Gisela thought it important to consider the multiple steps in the T4 life cycle as linked by common protein components. Thus, large protein complexes conducting recombination, repair, replication, and DNA packaging share common proteins or specific DNA substrates that tether them in a coordinated way (Mosig 1983). Similarly, mutations in gene *32* (encoding the single-stranded DNA-binding protein) display genetic connections to suppressor and enhancer mutations in several other genes (Mosig and Breschkin 1975; Mosig and Bock 1976; Mosig *et al.* 1977). She also recognized that the external environment was important to the malleability of viral reproduction, because selective pressures could guide viral replication through different replication origins (Lin 1988; Mosig 1994). She was particularly interested in the role of torsional stress in gene expression and DNA replication, especially in the choice of origins (Lin 1988). The differential use of replication origins depending on torsional stress also surfaced in her work on chloroplast replication in plants and especially in her work on *Chlamydomonas reinhardtii*.

Forays into evolution: Gisela was pleased when some of her later experimental observations led to insights into unusual evolutionary mechanisms. Her first such venture was a highly collaborative investigation into the process by which P2 prophages restrict T4-like phage infections by producing a protein that poisons gp32 (the single-stranded DNA-binding protein involved in numerous DNA transactions), a finding that "expands the repertoire that viruses use in the evolutionary struggle for survival" (Mosig *et al.* 1997, p. 79). Next, on the basis of her deep understanding of multiple mechanisms of recombination, she was able to interpret a local T2/T4 DNA sequence divergence of intimidating complexity (Gary *et al.* 1998; Mosig *et al.* 2001). The T2 and T4 versions of gene *56* show high DNA sequence identity in some regions but remarkable divergence in other regions, and this pattern could be understood as the accumulation of pairs of frame-compensating indels (with accompanying changes in amino acid sequence). The generating mechanism could be modeled as the product of ectopic recombination followed by the excision of loops in the resulting heteroduplex heterozygotes. This model has broad applicability. As they noted, it provides "one general and potentially mutagenic way to accomplish horizontal gene transfer . . . by a variation on a theme of homologous recombination [and has] obvious consequences for constructions of phylogenic trees based on sequence divergence, and the apparent different tempos of evolution of different genes in the same organisms" (p. 1471).

Revisiting plants: Perhaps because she grew up on a farm and had worked on petunia genetics for her doctorate, Gisela retained a strong interest in plant genetics and relished an opportunity to compare mechanisms of recombination and replication in chloroplasts and T4. Through a long collaboration with Robert Thompson, who was her graduate student and continued on the Vanderbilt research faculty, her lab initiated joint studies of Chlamydomonas and T4.

The 196-kb Chlamydomonas chloroplast genome contains two unique regions separated by a large inverted repeat (Gillham 1994), as do many other chloroplast genomes. The chloroplast genome includes some rRNAs and associated proteins, tRNAs, a subset of photosystem genes, and RNA polymerase subunits. Many of these genes are expressed in operons reminiscent of the chloroplast's prokaryotic ancestor. In addition, the Chlamydomonas chloroplast origins of replication, *oriA* and *oriB*, resemble *E. coli oriC*. Chlamydomonas *oriA* contains sequences similar to the *E. coli* DnaA box as well as promoters that can initiate primers for leading-strand DNA synthesis. These potential primers have stem-loop structures reminiscent of the RNA primers used in the *ColE1* origin of replication (Woelfle *et al.* 1993).

Mosig and Thompson characterized a Chlamydomonas topoisomerase activity that was similar to a bacterial gyrase (Thompson and Mosig 1985). Transcription from a specific set of genes was found to be regulated by tor-

sional stress (Thompson and Mosig 1987). Many of these genes were also under light–dark control. Therefore, environmentally conditioned gene regulation could be related to alterations of torsional stress and transcriptional regulation (Thompson and Mosig 1985, 1990). One of Gisela's graduate students, John Davies, looked at torsional stress during the developmental stage of chloroplast genomes in higher plants and found that the stress decreased as the chloroplasts developed (Davies *et al.* 1991).

An alternative mechanism for chloroplast DNA replication thought to depend on recombination was found by another of Gisela's graduate students, Mark Woelfle (Woelfle *et al.* 1993). Woelfle found that when Chlamydomonas was treated with sublethal doses of novobiocin (an inhibitor of type II topoisomerases), chloroplast origin initiation at *oriA* and *oriB* was initially inhibited. Later replication was initiated in only one region of the chloroplast genome in a novobiocin-insensitive manner. This process was reminiscent of the recombinational initiation of DNA replication in T4.

Gisela's interest in the evolution of the T4 genome also influenced her work on the chloroplast genome. Another graduate student, Wen-Hua Fan, discovered a new chloroplast-specific transposon, *Wendy*, which contained two inverted repeats surrounding two ORFs that contained protein motifs similar to some integrases and transposases (Fan *et al.* 1995).

Should we go to Russia? An International Congress of Genetics was scheduled for Moscow in 1978, and the Genetics Society of America (GSA) was raising funds to support the attendance of American geneticists. Genetics in the USSR was in bad shape because of the preceding decades of Lysenkoism, and it was dangerous to complain publicly about life in the USSR. There was discrimination against Jewish geneticists, and there was also concern that Israeli geneticists would be denied visas to attend the Congress despite the promises of the organizers and their government. The GSA Board of Directors had drafted a statement of concern and scheduled a debate on whether the meeting should be boycotted and financial support withdrawn. As a result, attendance was unusually large at the 47th GSA Annual Business Meeting held June 6, 1978, in Columbia, South Carolina. (J.W.D. sat next to Gisela during this meeting). Listening to the exchanges, Gisela showed agitation. Despite her distaste for public speaking, she was suddenly on her feet and talking. Both hands gripped the edge of the table and transmitted her trembling. She started out quietly and apologetically but her voice quickly gained firmness. She spoke briefly of her sense of isolation when living in communist East Germany and of the need for dialogue with the younger scientists working behind the Iron Curtain. How else would they learn disdain for authoritarian science and hear the truth about the quality of life in the West? How else to learn about cutting-edge genetics? (At the time, most USSR geneticists published in Russian in journals that had little or no outside impact, and the major Western journals were often inaccessible.) Finally, with a sharp exhalation, she sat down. The applause was polite but considerable. A vote was taken and Gisela's view prevailed by 103 to 3. Sadly, our concern about geneticists and Jews proved well founded. Word began to leak out that some Russians would not be allowed to attend the Congress, and stories about arrests also arrived. Visas were not provided to Israelis. (Some were provided just before the Congress was to begin, but too late to enable travel.) A number of prominent American Jewish geneticists then organized a boycott that was joined by a rather large fraction of the people who were on the program. As a result, the Congress was said to be disappointing for the Western participants, but Gisela's intervention nevertheless ensured that young Soviet scientists had at least some opportunity to interact with Western scientists and to hear other viewpoints, in contrast to the aborted 1937 Congress (Soyfer 2003).

Gisela as font of integrated knowledge and deep analysis: Gisela understood the complexity of T4 DNA transactions and never oversimplified her interpretation of experimental results. She read widely and acquired a deep knowledge and understanding of the context of experiments, both historically and in reference to potentially conflicting results. She also acquired an integrated knowledge of numerous organisms in addition to T4. She subscribed to numerous journals and summarized articles with detailed notes that she entered into a computerized database, not just for herself but also for friends and co-workers with interests in a wide variety of topics. She evangelically spread her knowledge and love of science to others whenever she could, often to biochemists and molecular biologists who had a much narrower view of the field. A phone call to Gisela was invaluable if one wanted to know the phenotypes of mutants, how these depended on growth conditions, or whether there was any genetic evidence for interactions between specific proteins. Her questions to others were rarely about published information, but almost always about their opinion of whether a work was reliable or a model reasonable. But while she was vigorous in her interrogations, she was also very supportive of new arrivals who were addressing meetings for the first time. Students have recounted looking directly at Gisela's approving face as a way to ease stage fright during talks. Gisela maintained an up-to-date database of all characterized T4 genes, including mutant phenotypes, restrictive hosts, functions, sizes, and relevant references; this was a key part of every T4 genome publication, including the most recent one dedicated to her memory (Miller *et al.* 2003).

Gisela as editor: For many years J.W.D. sent late drafts of his T4 papers to Gisela. She was consistently detailed in her criticisms. Most of these were comprehensible even as marginal scrawls, but sometimes a phone call

was needed to clarify one of her more cryptic comments. All the changes that she suggested were made or the sentence was reworked to say what had seemed to be perfectly clear in the first place. It was a privilege to receive such detailed criticisms because of the close attention they implied and the cleansing cautery that resulted.

Because of her exacting standards, Gisela's students often had to rewrite papers 30 or 40 times, which was daunting but always resulted in a better work. Over the years, she generously provided detailed critiques of papers and grant proposals to hundreds of people, particularly junior scientists. Despite the voluminous nature of her criticism, she always strove to be positive and enthusiastic about the work itself.

Gisela served with distinction on the Genetics Editorial Board from 1975 through 1989. At that time, the journal allowed authors to submit directly to an editor of their own choice. Some authors were incapable of appreciating Gisela's detailed help and chose a different editor, thus losing a chance to profit from her keen insights. She also served with distinction on the editorial boards of the Journal of Virology, Virology, and the Journal of Bacteriology and contributed substantially to both the writing and the editing of both the old and new testaments of the T4 bible (Mathews *et al.* 1983; Karam *et al.* 1994). As an editor, she was committed to helping people put their best foot forward and to getting their papers published, and she spent a lot of time helping scientists do just that.

Public service: Gisela served on numerous grant panels and chaired the National Institutes of Health Study Section on Microbial Genetics. She was an advisor in several capacities to the National Science Foundation, the American Cancer Society, and the National Institute of Environmental Health Sciences. She was often active in the affairs of the American Society for Microbiology (ASM), which last year established a graduate student travel award in her honor. As with most highly successful scientists, she chaired innumerable symposium sessions and helped to organize a number of meetings. She was a faithful attendant and speaker at the 1977–2001 Evergreen State College international phage biology meetings as well as at most Cold Spring Harbor phage meetings, Keystone DNA replication meetings, and GSA and ASM meetings. She often contributed from her own pocket to help her students attend meetings.

Awards: At Vanderbilt, Gisela received the Outstanding Graduate Teaching Award in 1989 and the Earl Sutherland Prize for Achievement in Research in 1995. She was elected a Fellow of the American Academy of Microbiology in 1994. She received the prestigious Humboldt Award for Senior Scientists in 1976.

A life beyond science: Gisela was deeply interested in the arts and was a scholar of classical music and a regular patron of the symphony who generously shared her tickets with students and friends. She loved to hike in Tennessee parks. She traveled the world, visiting exotic places with her sisters and brothers. She was dedicated to her family, all of whom lived in Germany. She brought a number of her nieces and nephews to Nashville, giving them the chance to study for a year at an American high school or college. Her extended family included her students, whom she taught that life includes more than science.

Fighting back from adversity: One evening a couple of decades ago, Gisela was walking home along a Nashville street when she was intercepted by a man with a pistol who demanded her purse. She had no memory of resisting, but he opened fire, striking both legs. He fled and was never apprehended. Gisela suffered a great deal of pain for several years. Surgeries gradually helped and one knee became free of pain. The pain subsided in the other knee but persisted for the rest of her life. It is a measure of her strength and determination that, as soon as she could, she resumed her habit of walking home from the lab each evening. Even during her final illness, she walked home after chemotherapy sessions, saying that the 25-min walk helped her to endure the treatment.

A small train station in Germany: One day in the late 1990s, Gisela was heading back to the States after visiting her family. The first leg out was a train from a small rural station. Gisela sat with a handful of hopeful travelers in the small waiting room. Looking around, she was discomforted to see the slogan "Ausländer Aus!" painted on a window. The other travelers seemed embarrassed but passive, perhaps the legacy of years of informers and secret police. Gisela dug into her purse. Unexpectedly, because she used cosmetics infrequently, she found a container of nail polish remover. She used this to obliterate "Foreigners Out!" and sat down, surprised at herself for this very public action. She was amazed when the waiting travelers clapped their hands in applause. (Gisela told J.W.D. this story with quiet pride.)

Gisela as teacher: Her breadth of research experience contributed to Gisela's excellence in teaching during her long career in the Department of Molecular Biology at Vanderbilt. Two of her memorable offerings were the undergraduate virus course, which was consistently oversubscribed, and the graduate course on Focal Topics in Genetics taught with Douglas Cavener. Gisela's teaching style was rife with visual examples that could include using Velcro strips to demonstrate complex recombinational mechanisms or paper models to demonstrate viral capsid morphogenesis. Focal Topics forced new graduate students to understand the latest literature in molecular biology at the sophisticated level necessary to pass the grueling preliminary exams in the Molecular Biology Department. Most of the younger members of the T4 community have also relied upon Gisela's mentoring at one time or another in their careers.

Departure: We have sketched several aspects of Gisela's

life and science but certainly not all; for instance, she carefully analyzed the expression of several T4 genes, sometimes uncovering unusually complex patterns. Three strikingly recurrent patterns in her career were her ability to remain unbound by popular views, her inclination to embrace and conquer complexity rather than flee from it, and the quality of her mentoring and assistance to her peers. A few years ago, Gisela discovered that she had metastatic ovarian cancer. An operation revealed its spread, but she was determined to undergo powerful chemotherapy, which ultimately provided her a few more productive years. At a meeting just months before her death, she was clearly exhausted, but she found the strength to tell N.G.N. all about her recent experiments with *oriE* and her future plans and to ask for help in testing her proposal that the RepEB protein loaded the helicase at this origin. When they talked in her last week, she was full of ideas for these experiments. Her students continued to visit her at the hospice to plan the experimental details.

A few years ago, Gisela was asked how she maintained her enthusiasm throughout her long career. She replied, "I have been so privileged to work on and teach something that interests me the most. It far exceeded any expectation that I had when I grew up. Is it surprising that I am enthusiastic about it?" (Snustad and Simmons 2000).

LITERATURE CITED

Breschkin, A. M., and G. Mosig, 1977 Multiple interactions of a DNA-binding protein in vivo. II. Effects of host mutations on DNA replication of phage T4 gene *32* mutants. J. Mol. Biol. **112:** 295–308.

Broker, T. R., 1973 An electron microscopic analysis of pathways for bacteriophage T4 DNA recombination. J. Mol. Biol. **81:** 1–16.

Broker, T. R., and A. H. Doermann, 1975 Molecular and genetic recombination of bacteriophage T4. Annu. Rev. Genet. **9:** 213–244.

Broker, T. R., and I. R. Lehman, 1971 Branched DNA molecules: intermediates in T4 recombination. J. Mol. Biol. **60:** 131–149.

Carles-Kinch, K., J. W. George and K. N. Kreuzer, 1997 Bacteriophage T4 UvsW protein is a helicase involved in recombination, repair and the regulation of DNA replication origins. EMBO J. **16:** 4142–4151.

Cox, M. M., M. F. Goodman, K. N. Kreuzer, D. J. Sherratt, S. J. Sandler *et al.*, 2000 The importance of repairing stalled replication forks. Nature **404:** 37–41.

Dannenberg, R., and G. Mosig, 1981 Semiconservative DNA replication is initiated at a single site in recombination-deficient gene 32 mutants of bacteriophage T4. J. Virol. **40:** 890–900.

Dannenberg, R., and G. Mosig, 1983 Early intermediates in bacteriophage T4 DNA replication and recombination. J. Virol. **45:** 813–831.

Davies, J. P., R. J. Thompson and G. Mosig, 1991 Intercalation of psoralen into DNA of plastid chromosomes decreases late during barley chloroplast development. Nucleic Acids Res. **19:** 5219–5225.

Epstein, R. H., A. Bolle, C. M. Steinberg, E. Kellenberger, E. Boy de la Tour *et al.*, 1963 Physiological studies of conditional lethal mutants of bacteriophage T4D. Cold Spring Harbor Symp. Quant. Biol. **28:** 375–392.

Fan, W.-H., M. A. Woelfle and G. Mosig, 1995 Two copies of a DNA element, 'Wendy', in the chloroplast chromosome of *Chlamydomonas reinhardtii* between rearranged gene clusters. Plant Mol. Biol. **29:** 63–80.

Gary, T. P., N. E. Colowick and G. Mosig, 1998 A species barrier between bacteriophages T2 and T4: exclusion, join-copy and join-cut-copy recombination, and mutagenesis in the dCTPase genes. Genetics **148:** 1461–1473.

Gillham, N. W., 1994 *Organelle Genes and Genomes*. Oxford University Press, New York.

Hutchinson, N., T. Kazic, S. J. Lee, C. Rayssiguier, B. S. Emanuel *et al.*, 1979 Late replication and recombination in the vegetative pool of T4. Cold Spring Harbor Symp. Quant. Biol. **43:** 517–523.

Karam, J., J. W. Drake, K. N. Kreuzer, G. Mosig, D. W. Hall *et al.* (Editors), 1994 *Molecular Biology of Bacteriophage T4*. American Society for Microbiology, Washington, DC.

Kosturko, L. D., and A. W. Kozinski, 1976 Late events in T4 bacteriophage production. I. Late DNA replication is primarily exponential. J. Virol. **17:** 794–800.

Kreuzer, K. N., and S. W. Morrical, 1994 Initiation of T4 DNA replication, pp. 28–42 in *Molecular Biology of Bacteriophage T4*, edited by J. Karam. American Society for Microbiology, Washington, DC.

Lin, G. W., 1988 Bacteriophage T4 DNA replication and transcription in phage and host type II topoisomerase mutants. Ph.D. Thesis, Vanderbilt University, Nashville, TN.

Luder, A., and G. Mosig, 1982 Two alternative mechanisms for initiation of DNA replication forks in bacteriophage T4: priming by RNA polymerase and by recombination. Proc. Natl. Acad. Sci. USA **79:** 1101–1105.

Marsh, R. C., A. M. Breschkin and G. Mosig, 1971 Origin and direction of bacteriophage T4 DNA replication. II. A gradient of marker frequencies in partially replicated T4 DNA as assayed by transformation. J. Mol. Biol. **60:** 213–233.

Mathews, C. K., E. M. Kutter, G. Mosig and P. B. Berget (Editors), 1983 *Bacteriophage T4*. American Society for Microbiology, Washington, DC.

Miller, E. S., E. Kutter, G. Mosig, F. Arisaka, T. Kunisawa *et al.*, 2003 Bacteriophage T4 genome. Microbiol. Mol. Biol. Rev. **67:** 86–156.

Mosig, G., 1960a Zur Genetik von Petunia hybrida. I. Die Selbststerilität. Z. Vererbungsl. **91:** 158–163.

Mosig, G., 1960b Zur Genetik von Petunia hybrida. II. Die Analyse von Genen der Anthoxanthin-und Anthocyanbildung in der Blüte. Z. Vererbungsl. **91:** 164–181.

Mosig, G., 1962 The effect of multiplicity of infection on recombination values in bacteriophage T4D. Z. Vererbungsl. **93:** 180–186.

Mosig, G., 1963a Coordinate variation in density and recombination potential in T4 phage particles produced at different times after infection. Genetics **48:** 1195–1200.

Mosig, G., 1963b Genetic recombination in bacteriophage T4 during replication of DNA fragments. Cold Spring Harbor Symp. Quant. Biol. **28:** 35–42.

Mosig, G., 1970a Recombination in bacteriophage T4. Adv. Genet. **15:** 1–53.

Mosig, G., 1970b A preferred origin and direction of T4 DNA replication. I. A gradient of allele frequencies in crosses between normal and small T4 particles. J. Mol. Biol. **53:** 503–514.

Mosig, G., 1983 Relationship of T4 DNA replication and recombination, pp. 120–130 in *Bacteriophage T4*, edited by C. K. Mathews, E. M. Kutter, G. Mosig and P. B. Berget. American Society for Microbiology, Washington, DC.

Mosig, G., 1987 The essential role of recombination in phage T4 growth. Annu. Rev. Genet. **21:** 347–371.

Mosig, G., 1994 Homologous recombination, pp. 54–82 in *Molecular Biology of Bacteriophage T4*, edited by J. D. Karam. American Society for Microbiology, Washington, DC.

Mosig, G., 1998 Recombination and recombination-dependent DNA replication in bacteriophage T4. Annu. Rev. Genet. **32:** 379–413.

Mosig, G., 2000 Incomplete genomes in small T4 particles, pp. 65–72 in *We Can Sleep Later: Alfred D. Hershey and the Origins of Molecular Biology*, edited by F. W. Stahl. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Mosig, G., and S. Bock, 1976 Gene 32 protein of bacteriophage T4 moderates the activities of the T4 gene 46/47-controlled nuclease and of the *Escherichia coli* RecBC nuclease in vivo. J. Virol. **17:** 756–761.

Mosig, G., and A. M. Breschkin, 1975 Genetic evidence for an additional function of phage T4 gene *32* protein: interaction with ligase. Proc. Natl. Acad. Sci. USA **72:** 1226–1230.

Mosig, G., and N. Colowick, 1995 DNA replication of bacteriophage T4 *in vivo*. Methods Enzymol. **262:** 587–604.

Mosig, G., and R. Ehring, 1968 Failure of incomplete T4 genomes to replicate under conditions of single infection. Virology **35:** 171–174.

Mosig, G., and R. Werner, 1969 On the replication of incomplete chromosomes of phage T4. Proc. Natl. Acad. Sci. USA **64:** 747–754.

Mosig, G., R. Ehring and E. O. Duerr, 1968 Replication and recombination of DNA fragments of bacteriophage T4. Cold Spring Harbor Symp. Quant. Biol. **33:** 361–369.

Mosig, G., R. Ehring, W. Schliewen and S. Bock, 1971 The patterns of recombination and segregation in terminal regions of T4 DNA molecules. Mol. Gen. Genet. **113:** 51–91.

Mosig, G., D. W. Bowden and S. Bock, 1972a *E. coli* DNA polymerase I and other host functions participate in T4 DNA replication and recombination. Nat. New Biol. **240:** 12–16.

Mosig, G., J. R. Carnighan, J. B. Bibring, R. Cole, H. G. Bock *et al.*, 1972b Coordinate variation in lengths of deoxyribonucleic acid molecules and head lengths in morphological variants of bacteriophage T4. J. Virol. **9:** 857–871.

Mosig, G., W. Berquist and S. Bock, 1977 Multiple interactions of a DNA-binding protein *in vivo*. III. Phage T4 gene-*32* mutations differentially affect insertion-type recombination and membrane properties. Genetics **86:** 5–23.

Mosig, G., A. Luder, A. Ernst and N. Canan, 1991 Bypass of a primase requirement for bacteriophage T4 DNA replication in vivo by a recombination enzyme, endonuclease VII. New Biol. **3:** 1195–1205.

Mosig, G., N. Colowick, M. E. Gruidl, A. Chang and A. J. Harvey, 1995 Multiple initiation mechanisms adapt phage T4 DNA replication to physiological changes during T4's development. FEMS Microbiol. Rev. **17:** 83–98.

Mosig, G., S. Yu, H. Myung, E. Haggard-Ljungquist, L. Davenport *et al.*, 1997 A novel mechanism of virus-virus interactions: bacteriophage P2 Tin protein inhibits phage T4 DNA synthesis by poisoning the T4 single-stranded DNA binding protein, gp32. Virology **230:** 72–81.

Mosig, G., J. Gewin, A. Luder, N. Colowick and D. Vo, 2001 Two recombination-dependent DNA replication pathways of bacteriophage T4, and their roles in mutagenesis and horizontal gene transfer. Proc. Natl. Acad. Sci. USA **98:** 8306–8311.

Séchaud, J., G. Streisinger, J. Emrich, J. Newton, H. Lanford *et al.*, 1965 Chromosome structure in phage T4. II. Terminal redundancy and heterozygosis. Proc. Natl. Acad. Sci. USA **54:** 1333–1339.

Snustad, P., and M. J. Simmons, 2000 *Principles of Genetics*, Ed. 2, pp. 427–428. John Wiley & Sons, New York.

Soyfer, V. N., 2003 Tragic history of the VII International Congress of Genetics. Genetics **165:** 1–9.

Stitt, B., and D. Hinton, 1994 Regulation of middle-mode transcription, pp. 142–160 in *Molecular Biology of Bacteriophage T4*, edited by J. D. Karam. American Society for Microbiology, Washington, DC.

Streisinger, G., R. S. Edgar and G. H. Denhardt, 1964 Chromosome structure in phage T4. I. Circularity of the linkage map. Proc. Natl. Acad. Sci. USA **51:** 775–779.

Thompson, R. J., and G. Mosig, 1985 An ATP-dependent supercoiling topoisomerase of *Chlamydomonas reinhardtii* affects accumulation of specific chloroplast transcripts. Nucleic Acids Res. **13:** 873–891.

Thompson, R. J., and G. Mosig, 1987 Stimulation of a Chlamydomonas chloroplast promoter by novobiocin in situ and in E. coli implies regulation by torsional stress in the chloroplast DNA. Cell **48:** 281–287.

Thompson, R. J., and G. Mosig, 1990 Light affects the structure of Chlamydomonas chloroplast chromosomes. Nucleic Acids Res. **18:** 2625–2631.

Vaiskunaite, R., A. Miller, L. Davenport and G. Mosig, 1999 Two new early bacteriophage T4 genes, *repEA* and *repEB*, that are important for DNA replication initiated from origin E. J. Bacteriol. **181:** 7115–7125.

Walker, Jr., D. H., G. Mosig and M. E. Bayer, 1972 Bacteriophage T4 head models based on icosahedral symmetry. J. Virol. **9:** 872–875.

Williams, K. P., G. A. Kassavetis, D. R. Henendeen and E. P. Geiduschek, 1994 Regulation of late-gene expression, pp. 161–175 in *Molecular Biology of Bacteriophage T4*, edited by J. Karam. American Society for Microbiology, Washington, DC.

Woelfle, M., A. R. J. Thompson and G. Mosig, 1993 Roles of novobiocin-sensitive topoisomerases in chloroplast DNA replication in *Chlamydomonas reinhardtii*. Nucleic Acids Res. **21:** 4231–4238.