Summary: Epigenetics—from Phenomenon to Field

D.E. GOTTSCHLING
Fred Hutchinson Cancer Research Center, Seattle, Washington 98109

When Bruce Stillman made his opening remarks at the 69th Cold Spring Harbor Symposium, one of the things he said he hoped to learn was a way to easily explain what "epigenetics" meant to his wife Grace. After a week of discussions, it became clear that such a request was akin to asking someone to define "family values"—everyone knew what it meant, but it had a different meaning for each person. Part of the reason for the range of opinions may be understood from the etymology of "epigenetics" as explained by David Haig: The word had two distinct origins in the biological literature in the past century and the meaning has continued to evolve. Waddington first coined the term for the study of "causal mechanisms" by which "the genes of the genotype bring about phenotypic effects" (see Haig). Later, Nanney used it to explain his realization that cells with the same genotype could have different phenotypes that persisted for many generations. I define an epigenetic phenomenon as a change in phenotype that is heritable but does not involve DNA mutation. Furthermore, the change in phenotype must be switch-like, ON or OFF, rather than a graded response, and it must be heritable even if the initial conditions that caused the switch disappear. Thus, I consider epigenetic phenomena to include the lambda bacteriophage switch between lysis and lysogeny (Ptashne 2004), pili switching in uropathogenic *Escherichia coli* (Hernday et al. 2003), position-effect variegation in *Drosophila* (Haynes et al.), heritable changes in cortical patterning of *Tetrahymena* (Frankel 1990), prion diseases (Wickner et al.), and X-chromosome inactivation (Huynh and Lee; Heard et al.).

This Symposium comes on the 100th anniversary of genetics as a field of study at Cold Spring Harbor Laboratory, making it a very timely occasion to consider epigenetics. Given this historical context, I thought it appropriate to provide an examination of epigenetics through the portal of previous Cold Spring Harbor Symposia. While this is the first Symposium dedicated to the topic, epigenetic phenomena and their study have been presented throughout the history of this distinguished series. The history I present is narrowed further by my limitations and likings. For a more complete and scholarly portrayal, I can recommend the more than 1000 reviews on epigenetics that have been written in the four years leading up to the 69th Symposium.

In presenting this chronological account, I hope to convey a sense of how a collection of apparently disparate phenomenon coalesced into a field of study that impacts all areas of biology.

All authors cited here without dates refer to papers in this volume.

A HISTORY OF EPIGENETICS AT COLD SPRING HARBOR SYMPOSIA

1941

In the 9th Symposium, the great *Drosophila* geneticist H.J. Muller described developments on his original "eversporting displacement," in which gross chromosomal rearrangements resulted in the mutant mosaic expression of genes near the breakpoint (Muller 1941). By the time of this meeting, he referred to it as "position effect variegation." It was well established that the affected genes had been transferred "into the neighborhood of a heterochromatic region," that the transferred euchromatic regions had been "partly, but variably, transformed into a heterochromatic condition— 'heterochromatized'," and that *addition* of extra copies of heterochromatic chromosomes "allowed the affected gene to become more normal in its functioning." This latter observation was an unexpected quandary at the time, which we now know to be the result of a titration of limiting heterochromatin components.

1951

In the 16th Symposium, a detailed understanding of the gene was of high priority. This may explain why little progress had been made on understanding position-effect variegation (PEV), though more examples were being discovered. However, the opening speaker noted that PEV would be an exciting area for future research (Goldschmidt 1952). Barbara McClintock noted that chromosomal position effects were the basis of differences in "mutable loci" of maize, and she speculated that the variation of mutability she observed likely had its roots in the same mechanisms underlying PEV in *Drosophila* (McClintock 1952).

1956

By the time of the 21st Symposium, McClintock's ideas about "controlling elements" had developed (McClintock 1957). Two were particularly relevant with regard to epigenetics. In the *Spm* controlling element system, she had uncovered variants that allowed her to distinguish between *trans*-acting factors that could "suppress" a gene (reduce or eliminate its phenotypic expression), and those factors that could mutate it. She also noted that some controlling elements could suppress gene action, not only at the locus where they had inserted, but also at loci that were located some distance on either side of them. Others were discovering this "spreading effect"

as well. J. Schultz presented a biochemical and physical characterization of whole *Drosophila* that contained different amounts of heterochromatin (Schultz 1957). While the work was quite primitive and the conclusions drawn were limited, the work represented early attempts to dissect the structure of heterochromatin and demonstrated just how difficult the problem would be.

1958

Two talks at the 23rd Symposium were landmarks with respect to our present day Symposium. First, R.A. Brink described his stunning observations of "paramutation" at the *R* locus in maize. If two alleles (R^{st} and R^{r}) with distinct phenotypes as homozygotes are combined to form a heterozygote, and this R^{st}/R^{r} plant is in turn crossed again, the resulting progeny that contain the R^{r} allele will *always* have an R^{st} phenotype, even though the R^{st} is no longer present (Brink 1959). However, this phenotype is metastable; in subsequent crosses the phenotype reverts to the normal R^{r} phenotype. He meant for the word paramutation "to be applied in this context in its literal sense, as referring to a phenomenon distinct from, but not wholly unlike, mutation." Second, D.L. Nanney went to great lengths to articulate "conceptual and operational distinctions between genetic and epigenetic systems" (Nanney 1959). In essence, he defined "epigenetics" differently from how it had been originally intended by Waddington (for details, see Haig). He found it necessary to do so to describe phenomenon he observed in *Tetrahymena*. He found evidence that the cytoplasmic history of conjugating parental cells influenced the mating type determination of resulting progeny. His definition encompassed observations made by others as well, including Brink's work on the *R* locus and McClintock's work noted in the 21st Symposium.

1964

Mary Lyon's recently proposed hypothesis of X-chromosome inactivation in female mammals (Lyon 1961) was of considerable interest at the 29th Symposium. S. Gartler, E. Beutler, and W.E. Nance presented further experimental evidence in support of it (Beutler 1965; Gartler and Linder 1965; Nance 1965). Beutler reviewed multiple examples of mosaic expression of X-linked genes in women, supporting the random nature of X-inactivation. From careful quantitative analysis of an X-linked gene product, Nance deduced that X-inactivation occurred before the 32-cell stage of the embryo.

1973

The 38th Symposium on Chromosome Structure and Function represented a return to the examination of eukaryotic chromosomes. Significant progress had been made studying prokaryotic and phage systems and consequently bacterial gene expression had dominated much of the thinking in the burgeoning field of molecular biology. An appreciation for chromatin (DNA with histones and nonhistone proteins) in eukaryotes was building, but it was unclear whether it played a role in chromosome structure or function or both (Swift 1974). Nevertheless, several groups began to speculate that posttranslational modification of chromatin proteins, including histones, was associated with gene transcription or overall chromosome structure (Allfrey et al. 1974; Louie et al. 1974; Weintraub 1974). There was only a hint of epigenetic phenomena in the air. It had been hypothesized that repetitive DNA regulated most genes in eukaryotes, partly based on the fact that McClintock's "controlling elements" were repeated in the genome. However, it was reported that most repeated DNA sequences were unlinked to genes (Peacock et al. 1974; Rudkin and Tartof 1974). From these observations, the idea that repeated elements regulated gene expression lost significant support from those in attendance. More importantly though, these same studies discovered that most of the repetitive DNA was located in heterochromatin.

1977

The 42nd Symposium demonstrated that in four years an amazing number of technical and intellectual advances had transformed the study of eukaryotic chromosomes (Chambon 1978). This included the use of DNA restriction enzymes, development of recombinant DNA technology, routine separation of proteins and nucleic acids, the ability to perform Southern and Northern analysis, rapid DNA and RNA sequencing, and immunofluorescent visualization of chromosomes. The nucleosome hypothesis had been introduced and mRNA splicing had been discovered. Biochemical and cytological differences in chromatin structure, especially between actively transcribed and inactive genes, was the primary interest at this meeting. However, most relevant to epigenetics, Hal Weintraub and colleagues presented ideas about how chromatin could impart variegated gene expression to an organism (Weintraub et al. 1978).

1980

The 45th Symposium was a celebration of Barbara McClintock's discoveries—Movable Genetic Elements (Yarmolinsky 1981). Mechanistic studies of bacterial transposition had made enormous progress and justifiably represented about half the presentations, while others presented evidence that transposition and regulated genomic reorganization occurred not only in maize, but also in other eukaryotes, including flies, snapdragons, *Trypanosomes, Ascobolus*, and budding yeast. In the context of this meeting, all observed variegated expression events were ascribed to transposition. Moreover, there was a reticence to seriously consider that "controlling elements" were responsible for most gene regulation (Campbell 1981), which led some to suggest that "the sole function of these elements is to promote genetic variability." In essence, the idea that heterochromatin was responsible for the regulated expression in PEV was called into question. With respect to future epigenetic studies, perhaps the most noteworthy discussion was the firm establishment of "silent mating cassettes" in *Saccharomyces cerevisiae* (Nasmyth et al. 1981; Rine et al. 1981).

1982

Leading up to the 47th Symposium, a general correlation had been established in vertebrate systems that the overall level of cytosine methylation in CpG DNA sequences was lower for genes that were transcribed than for those that were not. However, there were exceptions to this generalization, and more detailed analysis was presented that methylation of specific areas of a gene's promoter was most important (Cedar et al. 1983; Doerfler et al. 1983; La Volpe et al. 1983). Based on the restriction/modification systems of bacteria, it was thought that DNA methylation prevented binding of key regulatory proteins. Furthermore, it had been shown that DNA methylation patterns could be mitotically inherited in vertebrates, which led to the hypothesis that DNA methylation could serve as a means of transcriptional "memory" as cells divided through development (Shapiro and Mohandas 1983). Another major epigenetic-related finding was the identification of DNA sequences on either side of the "silent mating cassettes" in budding yeast that were responsible for transcriptional repression of genes within the cassettes—these defined the first DNA sequences required for chromosomal position effects (Abraham et al. 1983).

1985

The Molecular Biology of Development was the topic for the 50th Symposium and it too encompassed a number of important advances. Perhaps one of the most exciting developments was the overall awareness that fundamental molecular properties were conserved throughout evolution—e.g., human RAS functioned in budding yeast and homeobox proteins were conserved between flies and humans (Rubin 1985). New efforts to understand chromosome imprinting began with the development of nuclear transfer in mice (Solter et al. 1985). These studies revealed that parent-of-origin information was stored within the paternal and maternal genomes of a new zygote; it was not just the DNA that was important, but the chromosomes contained additional information about which parent they had passed through, and the information was required for successful development of an embryo. Part of the answer was thought to lie in the fact that differential gene expression was dependent on the parental origin of a chromosome (Cattanach and Kirk 1985).

There were a number of studies aimed at understanding the regulation of the bithorax complex. Notably, E.B. Lewis made special mention of the curious nature of known *trans* regulators of the locus; nearly all were repressors of the locus (Lewis 1985). Thus, the importance of maintaining a gene in a silenced state for many cell doublings was imperative for normal development. This contrasted with much of the thinking at the time—i.e., that gene activation/induction was where the critical regulatory decisions of development would be.

DNA transformation and insertional mutagenesis techniques had recently been achieved for a number of organisms. One particularly creative and epigenetic-related use of this technology came in *Drosophila*. A P-element transposon with the *white* eye color gene on it was created and "hopped" throughout the genome (Rubin et al. 1985). This provided a means to map sites throughout the *Drosophila* genome where PEV could occur.

This meeting also highlighted the first genetic approaches to dissecting sex determination and sex chromosome dosage compensation—in *Drosophila* (Belote et al. 1985; Maine et al. 1985) and *Caenorhabditis elegans* (Hodgkin et al. 1985; Wood et al. 1985).

1993

The 58th Symposium highlighted the celebration of the 40th anniversary of Watson and Crick's discovery. Part of the celebration was a coming out party for epigenetic phenomenon: New phenomena had been identified, molecular analysis of other phenomena had begun, and sufficient progress had been made in a number of systems to propose hypotheses and to test them.

In *Trypanosomes*, the family of variable surface antigen genes (VSGs) located near telomeres are largely silenced, with only one VSG expressed at a time. While this organism does not appear to contain methylated DNA, it was reported that the silenced VSGs contained a novel minor base: β-D-glucosylhydroxymethyluracil (Borst et al. 1993). This base appeared to be in place of thymidine in the DNA. Parallels between this base and cytosine methylation in other organisms were easy to draw; the modifications were important for maintaining a silenced gene. But how the base was introduced into the DNA or how it imparted such a function was unclear.

Progress had also been made in vertebrate epigenetic phenomena, including chromosomal imprinting and X-inactivation (Ariel et al. 1993; Li et al. 1993; Tilghman et al. 1993; Willard et al. 1993). It had become clear by this time that numerous loci were subject to imprinting in mammals—only one allele was expressed in diploid cells and expression was dependent on parental origin. The *Igf2-H19* locus was of particular interest, primarily because it contained two nearby genes that were regulated in opposing fashion. *Igf2* is expressed from the paternal chromosome while the maternal copy is repressed, whereas the paternal allele of *H19* is repressed and its maternal allele is expressed. Interestingly, methylated CpG was observed just upstream of both genes on the paternal chromosome. It was proposed that the differential methylation regulated access of the two genes to a nearby enhancer element; the enhancer was closer to, and just downstream of, *H19* (Tilghman et al. 1993). A mutually exclusive competition between the two genes for the enhancer was envisioned; when the *H19* gene was methylated, the enhancer was free to activate the more distant *Igf2* gene. Support for the idea that DNA methylation played a regulatory role in this process came from mouse studies. Mutation of the first vertebrate gene encoding a 5-methyl-cytosine DNA methyltransferase in embryonic stem cells showed that, as embryos developed, the paternal copy of H19 became hypomethylated and the gene became transcriptionally active (Li et al. 1993).

An important step in how ^{5Me}CpG mediated its effects came from the purification of the first ^{5Me}CpG DNA-binding complex (MeCP1) (Bird 1993). Not only did it bind DNA, but when tethered upstream of a reporter gene, MeCP1 caused the gene to be repressed. While this did not explain regulation at the *Igf2-H19* locus, it did provide a potential mechanism to explain the general correlation between DNA methylation and gene repression.

Genetic mapping over a number of years had identified a portion of the human X chromosome as being critical for X-inactivation. Molecular cloning studies of this X-inactivation center led to the discovery of the *Xist* gene (Willard et al. 1993), an ~17-kb noncoding RNA that was expressed only on the inactive X chromosome. The mouse version of *Xist* was surprisingly homologous in structure and sequence and held the promise of being an excellent model system to dissect how this RNA functioned to repress most of the X chromosome.

Two notable findings were described in *Neurospora* (Selker et al. 1993): first, cytosine DNA methylation was not limited to CpG dinucleotides, but could occur in seemingly any DNA context; and, second, the amazing phenomenon of repeat-induced point mutation (RIP). Sequences become "RIP'd" when there is a sequence duplication (linked or unlinked) in a haploid genome and the genome is put through the sexual cycle via conjugation. Two events occur: Both copies of the duplicated DNA pick up G:C → A:T mutations, and DNA within a few hundred base pairs of the RIP'd sequences becomes methylated. This double attack on the genome is quite efficient—50% of unlinked loci succumb to RIP, while tightly linked loci approach 100%—and readily abolishes gene function.

The *brown* gene in *Drosophila*, when translocated near heterochromatin, displays dominant PEV; the translocated copy can cause repression of the wild-type copy. In searching for enhancers and suppressors of this *trans*-inactivation phenomenon, Henikoff discovered that duplication of the gene located near heterochromatin *increased* the level of repression on the normal copy (Martin-Morris et al., 1993). While the mechanism underlying this event remained mysterious, it was postulated that the phenomenon might be similar to RIP in *Neurospora*, though it had to occur in the absence of DNA methylation, which does not take place in *Drosophila*.

Paul Schedl elucidated the concept of chromosomal "boundary elements" (Vazquez et al. 1993). The first were located on either side of the "puff" region at a heat shock locus in *Drosophila* and were defined by their unusual chromatin structure—an ~300-bp nuclease-resistant core bordered by nuclease-hypersensitive sites. It was postulated that such elements separated chromatin domains along the chromosome. Two in vivo assays supported this hypothesis: (1) When bordering either side of a reported gene, boundary elements effectively eliminated chromosomal position effects when the construct was inserted randomly throughout the genome; and (2) the boundary element was also defined by its ability to block enhancer function. When inserted between a gene promoter and its enhancer, the boundary element blocked the gene's expression. While not as well defined, the concept of boundary elements was also developing in other organisms, especially at the globin locus in mammals (Clark et al. 1993).

Budding yeast shined the light on a mechanistic inroad to chromatin-related epigenetic phenomena. It had already been established that the silencers at the silent mating type loci were sites for several DNA-binding proteins. Their binding appeared to be context dependent, as exemplified by the Rap1 protein, which not only was important in silencing, but also bound upstream of a number of genes to activate transcription (for review, see Laurenson and Rine 1992).

Over the years, numerous links had been made between DNA replication and transcriptionally quiescent regions of the genome. The inactive X chromosome, heterochromatin and silenced imprinted loci had all been reported to replicate late in S phase relative to transcriptionally active regions of the genome. In addition, it had been shown that the establishment of silencing at the silent mating type loci required passage through S phase, suggesting that silent chromatin had to be built on newly replicated DNA. Thus, there was great interest when it was reported that one of the silencers was found to be an origin of DNA replication and that its origin activity could not be separated from silencing function (Fox et al. 1993). Furthermore, mutants in the recently identified origin recognition complex (ORC) were found to cripple silencing (Bell et al. 1993; Fox et al. 1993).

The discovery that telomeres in *S. cerevisiae*, like those in *Drosophila*, exerted PEV opened another avenue for dissecting heterochromatic structure and its influence on gene expression. Reporter genes inserted near telomeres give variegated expression in a colony. The repressed state of the genes is dependent on many of the same gene products (*SIR2, SIR3*, and *SIR4*) as those required for silencing at the silent mating type loci. Several key aspects of the silent chromatin structure and the regulation of the variegated expression were described. It is worth noting that heterochromatin is defined cytologically as condensed chromatin, but silent chromatin in *S. cerevisiae* has never been visualized in this way. Nevertheless, because of similarities to PEV in *Drosophila*, there was enthusiasm to consider silent chromatin in yeast to be a functional equivalent of heterochromatin (described in Weintraub 1993).

A number of fundamental concepts came to light from the yeast studies. First, the importance of histone H3 and H4 became evident. In particular, the NH_2-terminal tail of histone H3 and H4 tails appeared to be directly involved in the formation of silent heterochromatin (Thompson et al. 1993). Specific mutations in the tails of these histones alleviated or crippled silencing and led to the notion that both the net charge of the residues on the tails and specific residues within the tails contributed to silencing. In addition, these early days of chromatin immunoprecipitation (ChIP) demonstrated that the lysines in the NH_2-terminal tail of histone H4 were hypoacetylated in regions of silent chromatin relative to the rest of the genome. Moreover, one of the histone mutants identified K16 of histone H4, which

could be acetylated, as critical for forming silent chromatin.

Telomeres appeared to provide the simplest system in which to develop an understanding of how Sir proteins mediated silencing. The concept of recruiting silencing proteins was being developed. Briefly, the telomeric DNA-binding protein, Rap1p, was found to interact with Sir3p and Sir4p by two-hybrid methods (described in Palladino et al. 1993). Thus, Rap1 could "recruit" these Sir proteins to telomeric region of the genome. There was evidence that Sir3p and Sir4p could bind to one another and, most importantly, Sir3p and perhaps Sir4p interacted with the tails of histone H3 and H4 (Thompson et al. 1993). Furthermore, overexpression for Sir3p caused it to "spread" inward along the chromatin fiber from the telomere, suggesting that it was a limiting component of silent chromatin and could "polymerize" along the chromatin (Renauld et al. 1993). Taken together, there appeared to be a large interaction network important for silencing; The Sir proteins initiated assembly at telomeric DNA, because of their interaction with Rap1p, and then "polymerized" from the telomere along the chromatin fiber, presumably by binding to the tails of histones H3 and H4.

Switching between transcriptional states in variegated telomeric expression appeared to be the result of a competition between silent and active gene expression (Aparicio and Gottschling 1994; described in Weintraub 1993). If the transcriptional activator for a telomeric gene was deleted, the gene's basal transcriptional machinery was insufficient for expression and the gene was constitutively silenced. Conversely, overexpression of the activator caused the telomeric gene to be expressed continuously; the gene was never silenced. In the absence of *SIR3* (or *SIR2* or *SIR4*) basal gene expression was sufficient, whereas increased dosage of *SIR3* increased the fraction of cells that were silenced. While a transcriptional activator could overcome silencing throughout the cell cycle, it was most effective when cells were arrested in S phase, presumably when chromatin was being replicated and hence most susceptible to competition. Somewhat surprisingly, cells arrested in G_2/M also could be easily switched, suggesting that silent chromatin had not yet been fully assembled by this time.

Silent chromatin in yeast was shown to be recalcitrant to nucleases and DNA-modification enzymes, suggesting that the underlying DNA was much less accessible relative to most of the genome (described in Thompson et al. 1993).

It also appeared that there was a hierarchy of silencing within the yeast genome: The telomeres were the most sensitive to perturbation, *HML* was next, and *HMR* was the least sensitive. In fact, when the *SIR1* gene was mutated, the normally completely silenced *HM* loci displayed variegated expression (Pillus and Rine 1989).

Lastly, Sir3p and Sir4p were localized to the nuclear periphery, as were the telomeres. It was proposed that the nucleus was organized such that the nuclear envelope provided a special environment for silencing (Palladino et al. 1993).

Schizosaccharomyces pombe also has silent mating cassettes that were suspected to behave similarly to the case in *S. cerevisiae*. However, in *S. pombe* there was an added twist to the story of mating type switching. In an elegant set of experiments, Amar Klar proposed how a "mark" is imprinted on one strand of DNA in a cell (Klar and Bonaduce 1993). The mark is manifested, after two cell divisions in one of the four granddaughter cells, as a double-stranded break that facilitates mating type switching. This yeast does not have any known DNA modifications (methylation, etc.); hence, a different type of mark was postulated to be left on the DNA strand.

1994

The 59th Symposium was on The Molecular Genetics of Cancer. The concept of epigenetic regulation in oncogenesis had begun to develop after the idea of tumor suppressor genes became established. While there had been a couple of studies supporting such a notion, an interesting twist to the story came in studies of Beckwith–Wiedemann syndrome and Wilms' tumor patients. Mutations in both types of patients had been mapped to a locus that included the imprinted *H19-IGF2* genes. Feinberg et al. discovered "loss of imprinting" (LOI) for these genes in affected patients: The maternal locus lost its imprint, *H19* was repressed, and *IGF2* was expressed (Feinberg et al. 1994). Thus, LOI, which in principle could occur elsewhere in the genome, could cause either biallelic expression and/or extinction of genes critical in oncogenesis.

1998

In the couple of years leading up to the 63rd Symposium on Mechanisms of Transcription, several important developments occurred that would impact the molecular understanding of several epigenetic phenomena. Histone-modifying enzymes were identified—specifically histone acetylases and deacetylases. Some of these enzymes proved to play critical roles in regulating gene expression and provided an entry into gene products that directly affected PEV and silencing. The tip of this iceberg was presented at the symposium (see Losick 1998). Molecular dissection of the Sir3p- and Sir4p-silencing proteins in yeast revealed the polyvalent nature of their interactions and how the network of interactions between all the Sir proteins, the histones, and various DNA-binding factors set up silent chromatin, as well as the molecular details of how various loci (telomeres, the rDNA, *HM* loci, and double-stranded breaks) could compete for the limited supply of Sir proteins. By crippling the ability of a specific locus to recruit silencing factors, Sir protein levels were increased at the other loci (Cockell et al. 1998). This provided direct evidence that principles of mass action were at work and explained how silencing at one locus could impact the epigenetic silencing at other loci—an idea originally put forth in studies on PEV in *Drosophila* (Locke et al. 1988).

Another finding explained how DNA methylation could regulate gene expression through chromatin. This came with the identification of protein complexes composed of MeCP2, which bind both methylated DNA and histone deacetylases (Wade et al. 1998). Methylated

DNA could serve as a point of recruiting deacetylases to a locus and thus facilitate silencing of nearby genes.

The concept of boundary elements was extended from *Drosophila* to mammals, with clear evidence provided at the β-globin locus, thus indicating that chromatin boundaries were indeed likely conserved in metazoans and perhaps all eukaryotes (Bell et al. 1998).

1999

The 64th Symposium on Signaling and Gene Expression in the Immune System provided evidence about how monoallelic expression arose and that it might be more widespread than previously thought. Monoallelic expression at the immunoglobulin loci had been obvious in lymphocytes for some time; it guaranteed the production of a single receptor type per lymphoid cell (Mostoslavsky et al. 1999). The allele to be expressed was chosen early in development, apparently at random; both alleles began in a repressed state, but over time one became demethylated. It was unclear how a single allele was chosen, but the phenomenon appeared at other loci, too, where the necessity of monoallelism was not obvious. For instance, only one allele of genes encoding the cytokines IL-2 and IL-4 was expressed (Pannetier et al. 1999).

2000

The most significant epigenetic-related talk at the 65th Symposium was the discovery that the Sir2 protein was a histone deacetylase (Imai et al. 2000). This was the only Sir protein that had clear homologs in all other eukaryotes and that regulated PEV. It seemed to be the enzyme primarily responsible for removing acetyl moieties from histones in silent chromatin. Furthermore, because it was an NAD-dependent enzyme, it linked the regulation of silencing (heterochromatin) to cellular physiology.

2003

The 68th Symposium on The Genome of *Homo sapiens* was an important landmark in genetics and, while there is still much genetic work to be done, the complete sequencing of this and other genomes signified that it was time to move "above genetics"—a literal meaning of epigenetics.

This historical account highlights several themes shared with many other areas of research. First, it demonstrates the episodic nature of advances in epigenetics. Second, as molecular mechanisms underlying epigenetic phenomenon began to be understood, it became easier to connect epigenetics to biological regulation in general. Third, it showed that people we now consider scientific luminaries had made these connections early on—it just took a while for most others to "see" the obvious.

THE 69TH SYMPOSIUM

Over the years a few general principles common to all epigenetic phenomena have been identified, and they serve to guide experimental approaches in the search for a mechanistic understanding. First, the differences between the two phenotypic states (OFF and ON) always have a corresponding difference in structure at a key regulatory point—i.e., form translates into function. Hence, identifying the two distinct structures, the components that compose them, and the compositional differences between them has been a primary task. Second, the distinct structures must have the ability to be maintained and perpetuated in a milieu of competing factors and entropic forces. Thus, each structure requires self-reinforcement or positive-feedback loops that ensure that it is maintained and propagated over many cellular divisions. In some cases, such as X-chromosome inactivation, this appears to be on the order of a lifetime.

Many of the mechanistic principles defined in the earlier symposia continued to be refined in the 69th Symposium, but there were also new developments. To put these new developments in context, it is important to note that two other discoveries had a major impact on epigenetics. One was the discovery of RNA interference and related RNA-based mechanisms of regulation. The other was the discovery of mechanisms underlying the prion hypothesis. Both of these fields advanced rapidly in the past decade, with some of the studies contributing to knowledge about chromatin-based epigenetics and others providing new perspectives about heritable transmission of phenotypes.

Below, I highlight but a fraction of the accomplishments from the Symposium that are advances I was able to appreciate. At the conclusion of this review, I will try to distill the most important concepts I took away from the meeting.

Propagating a Chromatin Mark

DNA methylation provides an easy-to-understand mechanism for propagating a phenotypic state as cells divide, but organisms such as yeast, flies, and worms propagate phenotypic states, even through meiosis, without DNA methylases. In these organisms, chromatin seems to be the likely structure that must be heritably propagated. Consequently, a mark on histones that persists and is duplicated in chromatin has become an attractive model for imparting an epigenetic mark (see Smith et al. 2002). While there have been a number of hypotheses put forth to explain how marked nucleosomes could be perpetuated, there have been no detailed mechanistic demonstrations of how this is accomplished.

The major issue hangs on the question: How is a mark in a parental nucleosome propagated to both sister chromatids following DNA replication? Histones H3 and H4 readily form a stable tetramer in vitro (Ruiz-Carrillo et al. 1979), and, in genetic analyses, modifications of these two histones play a critical role in silencing (Johnson et al. 1990; Thompson et al. 1994). The inherent structure of the nucleosome suggests that in contrast to the two heterodimers of histone H2A and H2B on either end of the nucleosome, the H3/H4 tetramer is likely to remain as a single unit (Luger et al. 1997), and in vivo isotope labeling experiments confirm the overall stability of the tetramer (Jackson 1988). Thus, during DNA replication it

seems that only one of the two sister chromatids will receive the histone H3/H4 "mark" of the parental nucleosome, while the other sister chromatid will receive new, naive histone H3/H4.

Data presented at the Symposium called these ideas into question and offered a model by which marks could be passed on to both sister chromatids (Nakatani et al.). Biochemical analysis of chromatin assembly factors that are known to be important for chromatin silencing in vivo revealed that these factors could bind to heterodimers, rather than the expected tetramers, of histone H3/H4 and assemble the dimers into mixed tetramers of H3/H4 in vitro. If this kind of mixing turns out to be true in vivo, then it provides a mechanism for passing equal amounts of marked parental histones to both sister chromatids. Further, it is implied in such a model that these mixed tetramers would employ a positive reinforcement mechanism by which the new histones would be rapidly modified to be identical to the parental histones.

Histone Modifications

Since 1995–1996, when the first histone-modifying enzyme genes were identified, there has been a wonderfully productive effort to identify enzymes responsible for adding or removing the myriad of posttranslational modifications observed on histones (see many of the papers and poster abstracts from the meeting). But just as we think we are approaching the end of this race, we are informed that the finish line has been moved—more than 20 new modification sites have been identified on histones in the past year (Zhang et al. 2003).

Nevertheless, some fundamental principles are understood. As observed in other multisubunit complexes, the modifications modulate interactions of the histones with other proteins, sometimes increasing the affinity of a protein for the nucleosome (methylated K9-histone H3 with HP1), or at other times diminishing an interaction (acetylation of histone H3 and H4 tails with Sir3 protein). In addition, there can be interdependence among modifications. For instance, methylation of K4 or K79 on histone H3 depends on the monoubiquitination of histone H2B (Osley 2004).

Another issue focuses on the number of methyl moieties on any given histone residue; primary amines of lysine can be mono-, di-, or trimethylated. In cases where the amine of lysine is required for binding with another factor, modification by even a single methyl group could interfere with achieving an interaction, as would the di- and trimethyl forms. However, if methylation is important to create an interaction, then only one methylated state may be relevant. With regard to this latter situation, R. Paro (pers. comm.) and Ringrose et al. (2004) provided beautiful evidence by in situ peptide competition that only trimethylated forms of K9 or K27 of histone H3 bound efficiently to Polycomb proteins in *Drosophila*. Furthermore, T. Jenuwein (Lachner et al.) provided evidence that the intermediate methylated states were also important in providing information on chromatin. For instance, using specific antibodies, he showed that heterochromatin in mouse cells reacted best with monomethyl K27-H3 and trimethyl K9-H3 and K20-H4. It will be worth following these findings with genetic and in situ competition experiments to determine whether all these specific marks are critical for heterochromatin formation and, if so, how the marks are made and how the marks mediate formation. Does each site contribute to the increased affinity of one protein (e.g., HP1) or do some of these marks help to lower the affinity for competing factors (e.g., euchromatic proteins in heterochromatin)?

The Histone Code Hypothesis

In considering histone modifications and their potential information content, there were many discussions about the "histone code hypothesis" (Jenuwein and Allis 2001). Most of those that I participated in, or overheard, were informal and rather lively. The proponents of the "code" cite examples such as trimethylation of K9 histone H3 and its greater affinity for the HP1 class of heterochromatin proteins (Jenuwein and Allis 2001), while those on the other side cite biochemical and genetic evidence that the net charge on the NH_2-terminal tail of histone H4, irrespective of the position of the charge, has dramatic effects on DNA binding or phenotype (Megee et al. 1995; Zheng and Hayes 2003).

Grunstein presented data that included genome-wide analysis of histone acetylation modifications and chromatin associated proteins using specific antibodies and ChIP-Chip in *S. cerevisiae* (Millar et al.). His focus was on the epigenetic switch associated with K16 acetylation for binding, or not binding, particular chromatin proteins—thus supporting the histone code hypothesis. Though not discussed, some of his data appeared to support reports from others that for much of the genome, there is no correlation between specific histone modifications and gene expression (i.e., all active genes have the same marks, and these marks are not present on inactive genes) (Schubeler et al. 2004; Dion et al. 2005). Taking all the results together, I suspect that both specific modifications *and* general net charge effects are used as mechanisms for regulating chromatin structure and gene expression.

Dynamic Silent Chromatin

I must confess that, based on static images of heterochromatin and the refractory nature of silent chromatin, I was convinced that, once established, a heterochromatic state was as solid as granite. Only when it was time for DNA replication would the impervious structure become relaxed. In thinking this way, I had foolishly ignored principles of equilibrium dynamics I learned in undergraduate chemistry. But these lessons were brought home again by studies of silent chromatin and heterochromatin, where it was shown that silencing proteins of yeast (Sir3) and heterochromatin proteins in mammalian cells (HP1) were in a dynamic equilibrium—proteins were rapidly exchanged between heterochromatin and the soluble compartment—even when the chromatin was in its most impervious state (Cheng and Gartenberg 2000; Cheutin et al. 2003). The realization of its dynamic qualities forced

a different view of how an epigenetic chromatin state is maintained and propagated. It suggests that, in some systems, the epigenetic state can be reversed at any time, not just during DNA replication. Hence, we can infer that mechanisms of reinforcement and propagation for silenced chromatin must function constantly.

Methylation of histones was widely held to be the modification that would indeed impart a "permanent" mark on the chromatin (for review, see Kubicek and Jenuwein 2004). In contrast to all other histone modification (e.g., phosphorylation, acetylation, and ubiquitination), there were no enzymes known that could reversibly remove a methyl group from the amine of lysine or arginine. Furthermore, removing the methyl group under physiological conditions by simple hydrolysis was considered thermodynamically disfavored and thus unlikely to occur spontaneously.

Those thinking that methylation marks were permanent had their belief system shaken a bit by several reports. First, it was shown that a nuclear peptidylarginine deiminase (PAD4) could eliminate monomethylarginine from histone H3 (Wang et al.). While this methyl removal process results in the arginine residue being converted into citrulline, and hence is not a true reversal of the modification, it nevertheless provided a mechanism for eliminating a "permanent" methyl mark.

Robin Allshire provided a tantalizing genetic argument that the *tis2* gene from *S. pombe* reversed K9 dimethylation on histone H3 (R. Allshire, per comm.). He may have been on the right track because a few months after the meeting, the unrelated LSD1 enzyme from mammals was shown to specifically demethylate di- and monomethyl K4 on histone H3 (Shi et al. 2004), thus reversing an "active" chromatin mark. Quite interestingly, LSD1 did not work on trimethylated K4; thus, methylation could be reversed during the "marking" process, but reversal was not possible once the mark was fully matured.

However, Steve Henikoff presented a way by which a "permanent" trimethyl lysine mark could be eliminated. He showed that the variant histone H3.3 could replace canonical histone H3 in a replication-independent transcription-coupled manner (Henikoff et al.). In essence, a histone that contained methyl marks for silencing could be removed and replaced with one that was more conducive to transcription. When total chromatin was isolated, histone H3.3 had many more "active" chromatin methylation marks (e.g., MeK79) on it than canonical histone H3 did.

In considering all these results, it seems that there may not be a simple molecular modification within histones that serves as a memory mark for propagating the silent chromatin state through cell division. Rather, there must be a more tenuous set of interactions that increase the probability that a silent state will be maintained, though they do not guarantee it.

Nuclear Organization

Correlations between nuclear location and gene expression have been made for many years (Mirkovitch et al. 1987). These observations began to drive the notion that there were special compartments within the cell where gene expression or silencing were restricted. It was argued that this organization was necessary to keep the complexity of the genome and its regulation in a workable order. This idea was supported by studies in *S. cerevisiae*, where telomeres are preferentially located at the nuclear periphery, as are key components of the silencing complex, such as Sir4 (Palladino et al. 1993). Mutations that released the telomeres or Sir4 from the nuclear periphery resulted in a loss of telomeric silencing (Andrulis et al. 2002; Laroche et al. 1998). Furthermore, artificially tethering a partially silenced gene to the periphery caused it to become fully silenced (Andrulis et al. 1998).

But in an insightful experiment, Gasser showed that if both the telomeres and the silencing complex were released from the periphery and free to move throughout the nucleus, telomeric silencing was readily established (Gasser et al.). Thus, there does not appear to be a special need for localizing loci to a compartment. This is more consistent with the findings that rapid movement of chromatin proteins on and off chromosomes can still mediate effective regulation such as silencing. Perhaps some of the localization is necessary to maintain high local concentrations of relevant factors under special—perhaps stressful—conditions.

Double-stranded RNA Mechanisms

Since the discovery that double-stranded RNAs could regulate gene expression and DNA transposition in plants, fungi, and *C. elegans*, some of the fundamentals have been worked out and follow a basic process (for review, see Tomari and Zamore 2005). First, double-stranded RNA (dsRNA) must be generated. In some cases it is introduced by humans (injected dsRNA or as transcribed inverted hairpin sequences). It could also be produced as the result of transcription of inverted repeats from viruses or transposons, from normal endogenous RNA with an inherent hairpin structure, or it may be generated by RNA-dependent RNA polymerases (RdRP) that reverse-transcribe "aberrant" RNAs within the cell (what constitutes "aberrant" is a matter for further research). Next, the dsRNA is cleaved into small (21–27-bp) RNA fragments by the RNase III enzyme Dicer. These small RNAs, which include small interfering RNA (siRNA) and microRNA (miRNA), are unwound and loaded into the RNA-induced silencing complex (RISC) or RNA-induced initiation of transcriptional gene silencing (RITS). At the heart of these complexes is the Argonaute protein, which binds the single-stranded RNA and uses it as a guide to direct the complex to RNA with the complementary sequence. Then, depending on the complex, the larger complementary mRNA may have its translation inhibited, it may be cleaved by an RNase H-related PIWI domain within Argonaute, or it may direct chromatin-mediated silencing of the gene producing the RNA. In systems where the RNA is cleaved, RdRP can initiate second-strand RNA synthesis. These new dsRNA molecules are processed further by Dicer to generate sec-

ondary siRNA, which can reinitiate RdRP synthesis and thus amplify the original signal in a cycle. However, it appears that some organisms do not utilize all the regulatory options outlined. For example, no RdRP homologs have been identified in *Drosophila* or mammals, where RNAi has a relatively short duration in dividing cells. Needless to say, there are still many details to be worked out in all these systems.

Many of the Symposium presentations addressed whether dsRNA-mediated pathways were involved in different epigenetic events. For instance, mutants in the RNAi pathways were tested to see if they affected PEV, cosuppression, or other phenomena. (Pal-Bhadra et al.; Robert et al.; Martienssen et al.).

In *S. pombe*, some of the differences were being assessed at the mechanistic level. While silencing at the mating type loci and heterochromatin within the centromere uses virtually all the same histone modifications and chromatin-silencing proteins, RNAi machinery does not appear to affect the mating loci, whereas the RNAi pathway is required for silencing within the centromeric heterochromatin (Allshire; Cam and Grewal). This appears to be the result of having two pathways for recruiting these chromatin-based silencing proteins. At the centromere, the silencing proteins are primarily recruited via the RNAi pathway (apparently via the RITS complex), while at the mating type loci, these proteins appear to be recruited via DNA-binding proteins, as occurs in *S. cerevisiae* (Cam and Grewal).

S. pombe also provided the best evidence that RNA, as it is being transcribed, serves as the recruitment site for RNAi-mediated chromatin silencing (Allshire). When a 280-nucleotide sequence was transcribed in vivo as an inverted repeat to form a 280-bp hairpin of RNA, this served to silence—through Swi6 and Me-K9 histone H3-based mechanisms—a second gene containing the 280 nucleotides at the 3′ end of its coding sequence. However, when a transcriptional terminator was inserted between the 5′ end of the coding sequence and the 280-base sequence, no silencing or chromatin-related proteins were detected at the gene. Thus production of a complementary RNA *in cis* appears to be necessary to recruit silencing machinery. This result leads to a bigger question. If the silencing machinery has been recruited, why is the message still being expressed?

A potential answer to this paradox may be provided by the discovery of DNA-dependent RNA polymerase IV, which is required for RNA-directed silencing via chromatin/DNA modification (Herr and Baulcombe). RNA Pol IV is distinct from its better known cousins—the DNA-dependent RNA polymerases I, II, and III, which transcribe ribosomal RNA genes, messenger RNA genes, and tRNA genes, respectively. RNA Pol IV may transcribe genes that are silenced via RNA-directed pathways to provide a cycle of reinforcement for the process. Whether it can transcribe within silenced chromatin remains to be determined, but it is worth noting that there are genes that can be expressed only when they are in heterochromatin (e.g., the *light* gene in *Drosophila*; Hearn et al. 1991).

Protecting the Genome

For some time, it has been thought that several of the epigenetic processes discussed at the Symposium (DNA methylation, heterochromatic repression, and RNAi machinery) were systems that were used to defend the cellular genome from foreign invaders such as viruses and transposons (Bestor and Tycko 1996). For host defense, transposition must be inhibited to protect the rest of the genome. While "spreading" of transposons may occur in the soma and even have phenotypic consequences, transposition in the soma of a metazoan is a "dead end." Successful reproduction for the transposon requires that it be passed on through the host's germ line. Consequently, transposition in germ cells is the only place where the transposon will be ultimately successful. If such a premise is correct, then the host must have a germ-line-specific defense system—but evidence for such a system has been lacking. This changed with the characterization of mammalian *DNMT3L* (Bestor and Bourc'his). This member of the DNA methyltransferase genes appears to be expressed in only germ cells of both male and female mice. Homozygous mutants of this gene are sterile and show rampant expression of two retrotransposons, LINE-1 and IAP, in germ cells. Intriguingly, meiotic catastrophe is observed in spermatocytes in the mutant, which may be the result of promiscuous transposition by these and other elements. The *DNMT3L* gene appears to be evolving rapidly in mammals, as if it may be "chasing" an ever-changing collection of transposons in the ultimate genetic "arms race."

Prions

Wickner provided an overview and criteria for defining a prion, and from his description it is clear they are part of the epigenetic landscape (Wickner et al.). In the simplest molecular sense, prions are proteins that can cause heritable phenotypic changes, by acting on and altering their cognate gene product. No DNA sequence changes occur; rather the prion typically confers a structural change in its substrate. The best-studied and -understood class of prions cause soluble forms of a protein to transition into amyloid fibers. In many cases, the amyloid form reduces or abolishes normal activity of the protein, thus producing a change in phenotype. Wickner defined another class of prions that do not form amyloid filaments. These are enzymes that require activation by their own enzymatic activity. If a cell should have only inactive forms of the enzyme, then an external source of the active enzyme is required to start what would then become a self-propagating trait, as long as at least one active molecule was passed on to each cell. He provided two examples and the expectation that this class of proteins will define a new set of epigenetic mechanisms to pursue.

Si presented preliminary evidence that a prion model may explain learned memory in *Aplysia* (Si et al.). Protein translation of a number of stored mRNAs in neuronal cells is important for the maintenance of short-term memory in this snail. He found that a regulator of protein translation,

CPEB (cytoplasmic polyadenylation element binding protein), can exist in two forms, and that the activated form of CPEB acts dominantly to perpetuate itself. It is still early days in testing this idea, but it offers an exciting new way to consider the mechanism by which memory in the brain occurs.

New Phenomenon

The description of a new and unexpected phenomenon always holds our imagination. One presentation in particular held my thoughts for weeks after the Symposium. Standard genetic analysis of mutant alleles of the *HOTHEAD* gene, which regulates organ fusion in *Arabidopsis*, revealed that normal rules of Mendelian genetics were not being followed (R. Pruitt, pers comm.). It was discovered that if heterozygous *HOTHEAD/hothead* plants self-fertilized and produced a homozygous *hothead/hothead* plant, and then this homozygous *hothead/hothead* plant was allowed to self-fertilize, the progeny from this homozygous parent reverted to a *HOTHEAD/hothead* genotype at a frequency of up to 15%. This stunning level of wild-type reversion produced an exact duplicate, at the nucleotide level, of the wild-type gene seen in the earlier generations. This reversion was not limited to the *HOTHEAD* locus—several other loci had similar frequencies of reversion to wild-type alleles. However, all the reversions required that the parent be homozygous *hothead/hothead*. The gene product of *HOTHEAD* did not offer an obvious explanation as to how this could occur, but discussions certainly suggested that an archival copy of the wild-type gene was transmitted, perhaps via RNA, through successive generations. While it could be argued that this phenomenon is outside the purview of "epigenetics" because of the change in DNA sequence, the heritable transmission of the putative archived copy does not follow normal genetic rules. Nevertheless, this phenomenon has enormous implications for the field of genetics, especially in evolutionary thinking.

Further Implications of Epigenetics in Human Health

There are abundant examples of how inappropriate gene regulation via epigenetic mechanisms can lead to human disease. This has led to a broader awareness about the field of epigenetics. For instance, it has resulted in pharmaceutical development of drugs that selectively inhibit many of the enzymes discussed at this Symposium (Curtin and Glaser 2003). It has also compelled some to develop a set of guidelines to aid health workers in assessing whether a disease has an epigenetic basis (Bjornsson et al.).

However, the basic science studies have led even further. I found it particularly exciting that A. Bird's continued work on MeCP2 mutants has led to a tangible explanation for Rett Syndrome—pointing straight to a metabolic defect in the brain (Bird and Macleod; A. Bird, pers. comm.). It was also a treat to have A. Klar close the Symposium by applying his creative energy to explain human psychosis based on epigenetic principles he originally developed by studying *S. pombe* (Klar). While their ideas wait further testing, I hope they are right.

There was an important presentation by R. Jaenisch, in which he described the successful nuclear transfer from a melanoma cancer cell into embryonic stem cells that ultimately led to the generation of mice (Jaenisch et al.). While the cells of these mice had the aberrant chromosomes of the original tumor and developed melanomas with a high penetrance and frequency, they also developed a wide spectrum of other types of tumors, though fibroblasts did not form tumors. These results set the stage to dissect the genetic and epigenetic events that are necessary for cancer to develop in different tissues. It holds great promise as a new tool for understanding oncogenesis.

CLOSING THOUGHTS

So what more needs to be done to understand epigenetic mechanisms? For the most part, we are still collecting (discovering) the components. Just as the full sequence of a genome has greatly facilitated progress in genetics, a clearer understanding for epigenetics will likely come when all the parts are known. It is encouraging to see the great strides that have been made in the last decade.

I confess that I cannot discern whether we are close to, or far away from, having an accurate mechanistic understanding about how epigenetic states are maintained and propagated. The prion-based phenomenon may be the first to be understood, but those that are chromatin-based seem the farthest off. The polyvalent nature of interactions that seem to be required to establish a silenced state on a chromosome increases the complexity of the problem. This is further compounded by the dynamic nature of silent chromatin. The ability to know more about movement of components in and out of chromatin structures requires application of enhanced or new methods for an eventual understanding. While ChIP has been important in establishing which components reside in a structure, it has temporarily blinded us to the dynamics.

I suspect that given the complexity, simply measuring binding and equilibrium constants between all the components and trying to derive a set of differential equations to simulate epigenetic switches may not be an effective use of resources, nor will it necessarily result in better comprehension. Rather, I speculate that a new type of mathematical approach will need to be developed and combined with new experimental measuring methods to eventually understand epigenetic events. Part of this may require development of in vitro systems that faithfully recapitulate an epigenetic switch between states. The in vitro system presented by Kingston (Fan et al.; R. Kingston, pers. comm.) provides the first steps toward this enormous task.

The idea of competition between two states in most epigenetic phenomena likely reflects an "arms race" that is happening at many levels in the cell, followed by attempts to rectify "collateral damage." For instance, silencing proteins may have evolved to protect the genome from transposons. However, because silencing proteins

work through the ubiquitous nucleosomes, some critical genes become repressed. To overcome this, histone modifications (e.g., methylation of K4 and K79 of H3) and variant replacement histones (H2A.Z) evolved to prevent silencing proteins from binding to critical genes. Depending on subsequent events, these changes may be co-opted for other processes—e.g., repression of some of the genes by the silencing proteins may have become useful (silent mating loci). The silencing mechanisms may have been co-opted for other functions as well, such as promoting chromosome segregation. And so it goes.

I look forward to having the genomes of more organisms sequenced, because this might lead us to understand an order of events through evolution that set up the epigenetic processes we see today. For instance, *S. cerevisiae* does not have RNAi machinery, but many other fungi do. By filling in some of the phylogenetic gaps between species, we may discover what events led to *S. cerevisiae* no longer "needing" the RNAi system.

Perhaps more than any other field of biological research, the study of epigenetics is founded on the attempt to understand unexpected observations, ranging from H.J. Muller's position-effect variegation (Muller 1941), to polar overdominance in the *callipyge* phenotype (Georges et al.). The hope of understanding something unusual serves as the bait to draw us in, but we soon become entranced by the cleverness of the mechanisms employed. This may explain why this field has drawn more than its share of lighthearted and clever minds. I suspect it will continue to do so as we develop a deeper understanding of the cleverness and as new and unexpected epigenetic phenomena are discovered.

In closing, I thank Bruce Stillman and David Stewart for organizing the Symposium, and all the faculty and staff at Cold Spring Harbor Laboratory for permitting epigenetic researchers a chance to share ideas and take a few more steps toward understanding.

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