failures or apparent malfunctions occurred in various elements of the total complex. Of course, the most serious was the failure of the launch-vehicle shroud to separate during the launch of Mariner III.

One anomaly, which demonstrates the need for exhaustive compatibility testing, occurred during the first attempted countdown for the launch of Mariner IV. At about the time the gantry was rolled back from the vehicle, it was reported that the magnetometer was producing abnormal data. It was decided to proceed provisionally with the countdown. When the launch was later postponed and the gantry returned to the vehicle, it was found that the anomaly had been caused by an interaction between the gantry and the magnetometer.

Even though schedules would not allow many of the life tests to begin until about the time of launch, these tests proved valuable in assessing the conditions aboard Mariner IV. By means of one such test, the cause of the malfunction of the plasma probe, which occurred about a week after launch, was determined. The plasma-probe unit used in the life test showed similar malfunction after operating for approximately the same amount of time, and the fault was found to be in a power-supply bleeder resistor. Because the location of the fault was known, it has been possible to partially interpret the plasma-probe data received after the rate of telemetry from the spacecraft was reduced from 33⅓ to 8⅓ bits per second.

After launch and during the flight of Mariner IV, the proof-model spacecraft was frequently operated to test various command sequences which had not previously been tried and which were needed to complete the mission. As changes in the operational plans were required during the 7½-month flight of Mariner IV to Mars, alternative procedures were tested and practiced by the operations team with the proof-model spacecraft. Simultaneously, the designers reexamined the "worst-case analyses," and failure modes were simulated.

Thus the interdependent tasks of the engineers and scientists in analyzing, testing, adjusting, rechecking, and sometimes compromising continued from the inception of the development until the mission was successfully completed.

The command to shift the spacecraft antennas did not cause the radiation counters, magnetometer, and cosmic-dust detector to be shut off, and so the spacecraft is continuing to respond to the interplanetary environment. The factors which will most substantially affect the future receipt on Earth of these interplanetary measurements are the performance of the spacecraft and its distance from the Earth. There is nothing inherent in the design of the spacecraft to preclude its operating for another 2 to 4 years. Figure 3 shows that the spacecraft will again be at a distance from Earth which will allow data reception during the summer of 1967. Perhaps at that time we will again be receiving the Mariner IV reports of interplanetary conditions.

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Heterochromatin

Heterochromatin provides a visible guide to suppression of gene action during development and evolution.

Spencer W. Brown

The history of heterochromatin is long and hoary. From the time of the 19th-century cytologists, odd assortments of densely staining flecks, blobs, rods, and agglomerations have been seen in the cell nuclei of various species of plants and animals. Modern insight began in 1928 when Heitz first saw the true relationship of these puzzling structures to the chromosomes, called them heterochromatin, and proposed that heterochromatin had special genetic attributes. The significance of heterochromatin in modern biology is based firmly on its relation to gene action in higher organisms and especially to the integration of gene action during development. Interest in heterochromatin extends from biochemistry and cytogenetics to clinical medicine.

Heitz and the Nuclear Cycle

The typical cell nucleus contains a small, well-defined organelle, the nucleolus, but the bulk of the nucleus appears to be an otherwise structureless maze of tiny dots and threads more or less uniformly dispersed in the nuclear sap and often forming a delicate reticulum. During division of the nucleus (mitosis), the nucleus itself disappears but is represented by the chromosomes. At the onset of mitosis, the dots and threads resolve themselves into elongate chromosomes, which gradually condense to form compact bodies grouped in the center of the cell. At this point, each chromosome splits lengthwise and the two halves separate from each other toward opposite ends of the cell. A specific chromosome region, the centromere, is responsible for the movement of the chromosomes during separation. At the two ends of the cell, the condensation process is reversed; the

The author is professor of genetics at the University of California, Berkeley.

chromosomes gradually unravel to form new nuclei. The nucleolus is usually not maintained during a divison cycle; it shrinks and disappears about midway through the condensation process and is restored gradually during the unravelling phase.

By carefully following chromosomes through division cycles Heitz (*1*, *2*) proved that the puzzling structures which stained densely in the nucleus were simply specific chromosomes or chromosome regions that did not decondense or unravel like the rest of the chromosome material during the formation of the new nuclei. The dense structures remained visible in the nucleus until near the onset of the next division; they then loosened briefly only to condense again quickly, ahead of the remaining chromosome material. They could thus be traced during the condensation and unravelling processes but appeared like any other part of the chromosome during its most compact phase, just before and after it split lengthwise. Heitz thus recognized two classes of chromosome material, the *euchromatin*, which underwent a typical cycle of condensation and unravelling, and the *heterochromatin*, which maintained its compactness in the nucleus.

Heitz used the same method of highly refined sequential analysis to show that the nucleolus was not a part of the chromosomes but was formed or organized at a specific site in the chromosome set. In most organisms with two sets of chromosomes, one of maternal and the other of paternal origin, there are two such organizing regions, one within each set of chromosomes and at exactly equivalent or homologous sites. The heterochromatic zones, though usually more than two in number, were also precisely placed at homologous sites in the two sets. Thus, both the region of nucleolar organization and the heterochromatin were seen to conform to the highly specific, stable patterning otherwise known to be characteristic of the hereditary machinery.

Heitz believed heterochromatin to be genetically inert. He based this proposal on the ideas of earlier cytologists, such as Roux and Boveri, who thought that the chromosomes were genetically active only in the nucleus itself, not during the division process. If then heterochromatin maintained its divisional compactness in the nucleus, it could not be genetically active.

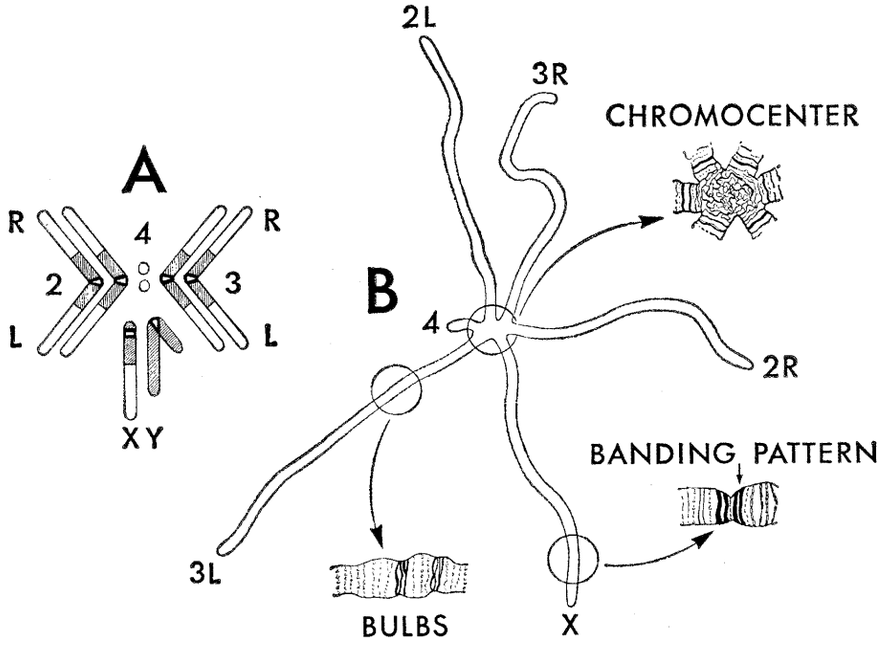

Fig. 1. Chromosomes of the fruit fly, *Drosophila melanogaster*. (*A*) Diagram of chromosomes in division in the body cells of the male; heterochromatic regions are shaded; the centromeres are indicated by clear zones set off by heavy lines; chromosome 4 has been left uncharted. The chromosomes regularly associate in pairs at mitotic divisions of the fruit fly and its relatives, but such pairing occurs rarely in other organisms. (*B*) Diagram of the giant chromosomes. The homologous chromosomes are intimately paired along their lengths; the centromeres and centric heterochromatin are combined in the chromocenter; each chromosome arm radiates independently from the chromocenter. In the X chromosome, banding pattern is shown near the region of an eye-color gene (small arrow). In the left arm of chromosome 3 are bulb-like structures which swell at a specific time in development. (The giant chromosomes are over 100 times as long as those in mitosis.)

The Cytological Picture

Since Heitz's earlier work, heterochromatin has proved widespread in species of plants and animals (*2*). It stains typically with the usual dyes used for work with chromosomes, including Schiff's reagent in the well-known Feulgen test for DNA. Heterochromatin tends to occur in similar regions in the different chromosomes of a species, most often immediately next to the centromere (the centric heterochromatin), at the ends of the chromosomes, and in the vicinity of the nucleolus organizer. Heterochromatin is frequently a large constituent of sex chromosomes and of the extra, or supernumerary, chromosomes which occur in some species. Individual entities of heterochromatin sometimes combine with each other to form larger structures, often called chromocenters; these combinations sometimes appear as amorphous agglomerations in which the individual components are no longer identifiable.

In the individual chromosome, heterochromatin frequently occurs in large blocks or segments, but these may be interrupted by pieces of euchromatin, and, conversely, small bits of heterochromatin may occur anywhere in the euchromatin. The euchromatin itself may frequently be seen to contain numerous small bead-like structures called chromomeres; a tiny bit of heterochromatin thus may often be superficially indistinguishable from a euchromatic chromomere.

The differentiation between euchromatic and heterochromatic segments is usually studied only in those few tissues or species which are particularly well suited to cytological examination. We know as yet relatively little about how the chromosomes may vary during development and from tissue to tissue.

There are, however, some striking examples of tissue-to-tissue variation; one of the best occurs in the familiar fruit fly, *Drosophila melanogaster* (*2*, *3*). As shown schematically in Fig. 1*A*, the chromosomes of *Drosophila* consist of two sex chromosomes, the X and the Y (XX in the female, XY

in the male) and three others, or autosomes, which occur as pairs of homologues in both sexes. The two large autosomes and the X have large blocks of centric heterochromatin, whereas the Y consists almost entirely of heterochromatin. The dot-like autosome, chromosome 4, has proved too small for such analysis. The heterochromatic blocks do not appear during early stages of development; they do show up in later cell divisions in many but not all of the cells examined (*4*).

In the giant salivary gland chromosomes, the picture is entirely different (Fig. 1*B*). Here the chromosomes are greatly extended and thickened, and each chromosome is closely united with its homologue. The chromosomes are marked by densely staining structures, called bands, which are separated by very weakly staining gaps, or interbands. The characteristic banding patterns have permitted exact identification of each small segment of the chromosomes (*2*). The heterochromatin, so prominent in other tissues, shows up as only a very few bands at the base of each chromosome arm (*3, 4*). The centric heterochromatin of all the chromosomes is clumped together to form a chromocenter in which the few bands present are usually highly distorted and difficult to analyze. The Y chromosome is entirely included in the chromocenter and can be charted only by the most expert *Drosophila* cytologists. In the giant salivary gland chromosomes there probably is only about 1/20 as much heterochromatin as there is in the mitotic chromosomes; such estimates are made on a length-for-length basis and do not include the small 4th chromosome or the Y. For the Y chromosome, the change in proportional size would be much greater.

Several authors have suggested that heterochromatin, as commonly observed in typical cells and division cycles, owes its bulk to accessory material rather than to differential condensation. According to such concepts, the true lengths of the various chromosome segments are revealed only when the chromosome is quite extended, as in the giant chromosomes. The relatively greater bulk of heterochromatin in the mitotic chromosomes must therefore be due to a "ballast" substance (*4*). According to Dobzhansky (*5*), large euchromatic segments of the giant X chromosome of *Drosophila pallidipennis* may disappear into the chromocenter in some of the nuclei of the salivary gland. Thus the lengths of the giant chromosomes may not represent true proportions. Since heterochromatin is not visible in the nuclei during embryonic stages, the chromosomes must lack the accessory material. On this basis, the Y chromosome, for example, would be barely visible during division; but no such striking changes in the embryonic chromosome sets have been reported. Quite recently Rudkin (*6*) has reported the probably true basis for the discrepancy between the giant and the mitotic chromosomes: the heterochromatic segments fail to synthesize DNA during the formation of the giant chromosomes.

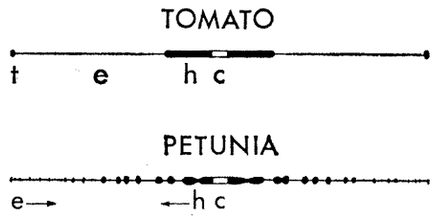

Fig. 2. The tomato and the petunia belong to the same family of plants, the Solanaceae. The tomato chromosome is sharply differentiated into distal euchromatin (*e*) and heterochromatin (*h*) adjacent to the centromere (*c*); each chromosome arm terminates in a small bit of heterochromatin (*t*). The petunia chromosome is tapered. Heterochromatin (*h*) is obvious near the centromere (*c*) and euchromatin (*e*) near the ends, but the mid-regions appear to be mixtures of euchromatin and gradually decreasing bits of heterochromatin. The terminal elements are not conspicuous in the petunia.

Differences between related organisms show how quickly the evolutionary processes can result in rearrangement of the heterochromatin. In the tomato, large blocks of centric heterochromatin are clearly distinguishable. In another plant of the same family, the petunia, the chromosomes are tapered structures (Fig. 2). From the end to the centromere, the chromomeres gradually increase in size, and it is impossible to say where the euchromatin of the petunia ends and the heterochromatin begins.

At specific stages in the development of some organisms (*2, 7*), and following certain environmental changes (*2*), the heterochromatic chromosomes will appear to be quite obviously *less* condensed than the others during the division cycle. A possible explanation of this "negative heterochromatin" is that the condensation of the heterochromatin has become quite unsynchronized with the division cycle.

Heterochromatin Defined

Heitz's definition of heterochromatin as a substance breaks down because the material is not present as such at all stages of development (*4*). Its absence in the early embryonic stages of the fruit fly is particularly significant; if it were an inherited entity, its presence at this time would certainly be expected. As we shall see later, probably all chromosome regions are potentially capable of becoming heterochromatic, but in most organisms only certain segments will usually so respond during development.

We must, therefore, regard euchromatin and heterochromatin as states rather than substances. Since Heitz's definitions were descriptive and discriminatory rather than dogmatic and inclusive, we can continue to use them equally well for the changes in state observed inside the cell nucleus. A heterochromatic segment of a chromosome thus becomes a region which is regularly and frequently observed to become heterochromatic. Further precision will be possible only when we have learned more about nuclear changes in development. In the meantime, numerous descriptive terms are available for puzzling and borderline cases.

The Genetic Corollary

As early as 1914, the geneticist H. J. Muller had become concerned about the seeming genetic anomalies of the Y chromosome. By the time Heitz's concepts were published, sufficient information had become available on the distribution of the genes in the chromosomes of the fruit fly to indicate that the heterochromatin had far fewer genes per unit length than the euchromatin (*3*). The Y was necessary, but only for the fertility of the males, and development proceeded quite normally without it until sperm formation. In an intensive study of the problem, Cooper (*4*) found that if the comparison was made on the basis of lengths in the salivary gland chromosomes, in which the heterochromatin is much reduced, the proportion of genes in the euchromatin and the heterochromatin was the same. Khush, Rick, and Robinson (*8*) have recently reported the first gene to be localized in centric heterochromatin in tomato. Such studies show that heterochromatic blocks are not

completely inert. The genes present may be located on small bits of intercalated euchromatin, but other explanations are certainly possible.

A characteristic of euchromatin is its ability to undergo crossing over. During the reduction divisions (meiosis), which in animals occur immediately prior to formation of the sperm and egg, homologues from the two sets of chromosomes pair together and exchange segments. Although the exchange mechanism itself is not known as yet, the results of such exchange have been studied in detail in many plants and animals. Very roughly, the frequency of exchanges is proportional to chromosome length; up to a certain limit, the longer the segment between two genes, the more apt the genes are to be reshuffled by crossing over. The genes lying on opposite sides of the long blocks of centric heterochromatin in *Drosophila* tend to stay together; on a length-for-length basis they undergo exchange at a very low rate (*3*).

Many cytologists believe that such an exchange results in a visible node or chiasma that seems to interconnect the two paired chromosomes. In the tomato, chiasmata have not been found in the centric heterochromatin; this observation may, in part, be explained away by the possibility that those chiasmata which were formed in the heterochromatin later slipped down toward the ends and into the euchromatin. However, Barton's test (*9*) for crossing over in the one exclusively heterochromatic chromosome arm of the tomato gave completely negative results. Claims have been made that in a few species chiasmata frequently occur in heterochromatin exceedingly difficult to analyze at the necessary division stages, but these claims have yet to be confirmed by genetic tests. It is generally accepted that crossing over provides new combinations of genes on which natural selection may act. The relatively few genes occurring in heterochromatin would require, on a gene-for-gene basis, a vastly reduced rate of crossing over. There is some evidence that the crossing over which does occur in the heterochromatin of *Drosophila* is of an exceptional type (*2*).

One striking anomaly remains. In a few organisms, including the fruit fly, exchanges also occur in mitotic divisions during the development of the body as well as in meiosis. Variant patches of tissue are sometimes detectable as a result of these exchanges. Stern, the original discoverer of mitotic exchanges, found that most of them occurred near the centromere in the fruit fly, and Kaplan, Brosseau, and Walen have each localized a high proportion in the heterochromatin (*10*). Chromosomes with exchanges in heterochromatin, and which are recoverable through the sperm and egg, are presumably produced by crossing over in the mitotic divisions of the gonad (*2*). In the fruit fly, there are frequent deleterious genes; those on the maternal set are covered by normal ones on the paternal set and vice versa. Because the mitotic exchanges would expose these deleterious genes, such exchanges seem to be of no developmental advantage. They may possibly be the result of the influence of some other, necessary, developmental process on the heterochromatic state, or perhaps the consequence of selection for at least a few exchanges in heterochromatin.

Position Effect

The influence of chromosome sectors is pervasive. If a euchromatic segment is artificially relocated next to heterochromatin, its genes will be depressed in their function. The action of a gene normally occurring in heterochromatin will also be depressed if the gene is moved next to euchromatin (*3, 11*). Because gene action is altered in both types of relocation, such effects are undoubtedly a reflection of the adaptation of the gene to its original milieu and, therefore, are not evidence of the negative influence of heterochromatin per se. This "position effect" on genes often expresses itself as a variegation. When one of the genes responsible for the red eye color of the fruit fly is so affected, the eye will appear as a mosaic of red and white sectors; since white is the typical expression when this gene is otherwise seriously damaged, it has been concluded that the heterochromatin is inactivating the gene in the white sectors. Schultz (*12*) and others proved that additions of still more heterochromatin elsewhere in the chromosome complement, such as an extra Y chromosome, surprisingly tended to reverse the depressing effect of heterochromatin on euchromatic genes.

In a remarkable series of experiments, Becker (*13*) showed how developmental control of gene action was disturbed by position effect. With x-rays, Becker induced mitotic exchanges of eye-color genes at carefully timed intervals during development. Since each affected sector was the progeny of a single cell containing an exchange product, it was possible to chart the normal developmental sequence of the tissue composing the eye. Next Becker found that position effect produced large mosaic sectors in the variegated eyes. These large sectors corresponded precisely in size with those produced by x-ray treatment early in development and were much larger than those appearing after treatments later in development. Thus position effect simply acted to disturb the normal preconditioning of the gene during development, long before the gene would be called upon to perform. However, the disturbance was not always absolute, and the eye-color genes in all the cells of some, but not all, sectors could be prevented from functioning normally by changes in the environment imposed at later developmental stages. All the cells in a sector would either produce or fail to produce pigment. This mechanism can be compared with electric light switches that may be turned either off completely, or on, or so barely at the "on" position that a slight jar will turn them all off.

Gene Action and Development

Our present picture of gene action comes almost exclusively from microorganisms (*14*). It is a verbally simple one. The base sequence of DNA is transcribed into that of a complementary RNA, the messenger. The messenger RNA leaves the DNA and attaches to a ribosome, where molecules of transfer RNA, each bearing a specific amino acid, translate the messenger sequence into an amino acid sequence and thereby construct a protein. The base sequence is translated in triplets; three nucleotide pairs must be present in DNA for each amino acid in the final product.

In microorganisms the activity of genes responsible for making certain enzymes can be tested precisely by testing for the presence or absence of the enzyme itself. Certain genes remain continuously active; others become active only in the presence of the metabolite on which their enzyme will act. The most famous model explaining this response of the genes themselves to metabolic demand is that of Jacob and Monod (*14*).

It has been known for some time that cell nuclei in multicellular organisms also deliver RNA to the cytoplasm, but only recently has this production been correlated with development. In the giant chromosomes of the diptera, "puffs" are formed by specific bands or small chromosome segments, such as the bulbs of chromosome 3 (Fig. 1*B*). On puffing, the chromosome segment swells and the bands tend to become indistinct. The difference between the puffed and unpuffed bands is thus superficially similar—expanded versus condensed—to the difference between euchromatin and heterochromatin. In lengthy series of experiments, Becker, Mechelke, Beerman, Clever, and others have discovered some remarkable features of the puffing process (*15*). RNA metabolism is carried on actively at the site of the puff. The puff appears and disappears at precise developmental stages, most often preceding molting, and the insect molting hormone, ecdysone, can be used to initiate the schedule artificially. A puff which has gone through one cycle can be made to do so again by transplanting the salivary gland into a younger larva. In certain diptera, tissues other than the salivary gland also have giant chromosomes, and these chromosomes show their own series of puffings. The entire picture is exactly what would be expected if genes in higher organisms were also active via messenger RNA and this action were precisely integrated with development.

Controlling mechanisms in lower and higher organisms have certain genetic aspects in common (*16*), but the molecular machinery responsible for turning genes on and off is unknown in either instance. The DNA of higher organisms is combined with proteins, a large part of which are histones. The histones and their combinations with nucleic acids have been the subject of a recent international conference from which emerged as many problems as facts. The Stedmans had earlier suggested that histones function as gene inhibitors which permit differential gene action and therefore the formation of different cell types during development. Bonner and Huang showed that histones can block the formation of messenger RNA by DNA (*17*). According to Bradbury and Crane-Robinson, the mode of attachment of histone to nucleic acid is not understood; the general belief, however, that this attachment is largely nonspecific led Zubay to question the possibility that histones function in the regulation of specific genes (*17*). The recent work of Huang and Bonner (*18*) has modified these concepts considerably. The molecules of chromosomal histones occur in large aggregates and one molecule of an unusual type of RNA is associated with each aggregate. The RNA could guide the histone to a specific site of chromosomal DNA where repression of genetic activity could then occur.

Other work indicates that there is a superabundance of DNA at each gene locus in higher organisms and that most of the RNA formed inside the nucleus is destroyed there rather than released to the cytoplasm to act in protein synthesis (*19*). Such observations would make sense if the genes in higher organisms were required to build complex machinery for their own control.

It is onto this still-unresolved picture of the individual locus that heterochromatization must be superimposed. But complex regulative devices at individual loci could offer a variety of possibilities by which adjacent loci could be interconnected and their region of the chromosome kept in a condensed state. The physical basis of heterochromatin may consist merely of relatively simple interconnections or interactions rather than additions or losses of chromosome material. Certainly a consideration of the superregulation undergone by every chromosome as it condenses during cell division is worthwhile here; the divisional changes are temporary, and they must be superficial because, as Becker and others have shown, both heterochromatization and gene conditioning will survive them. Yet the onset of cell division is sufficient to turn off most of the RNA metabolism (*20*).

If we picture the chromosome as sustaining a hierarchy of regulatory devices, heterochromatization, which usually involves regions of chromosomes, must lie someplace between those devices controlling individual gene loci and that which is recognizable in the changes undergone by all the chromosomes during cell division. These mechanisms may all have a similar physical basis, and all were probably developed coevally during the early evolution of the multicellular organism.

Approached from other angles, the problem of developmental genetics appears no simpler. Development is a remarkably precise and complex process, and equally precise and complex tools are undoubtedly required for its achievement. We expect eye-color genes to show their effects in the eye, but, as Stern, Hannah-Alava, Tokunaga, and others have found in the fruit fly, there are other, much more subtle patterns of differentiation also controlling gene expression (*21*).

McClintock, Brink, and their colleagues have offered overwhelming evidence that hereditary factors control gene action in maize. Whether or not these controlling factors are, as Brink (*22*) has indicated, superimposed on the normal machinery, the wide array of events precipitated by alterations in these systems, ranging from chromosome breakage to changes in strength of gene action and in the timing of gene action during development, cannot be other than a reflection of the truly frightening dimensions of developmental genetics. That the behavior of heterochromatin is also under close control and may be directly involved in some of the systems studied was discussed by McClintock (*23*) in the earlier phases of the work and more recently by Brink (*22, 24*). The regulative factors are, however, usually too small for direct microscopic study, and must be recognized by their effects on other genes.

Facultative Heterochromatization

Heterochromatin may be studied least ambiguously when formed *de novo* during development, and we may contrast the two kinds in this regard. In *constitutive* heterochromatization both the homologous chromosomes, one maternal, the other paternal, respond in the same way during development. In *facultative* heterochromatization, the two homologous chromosomes differ; one becomes heterochromatic during development, and the other remains euchromatic. Thus facultative heterochromatization provides an unparalleled opportunity for studying the same genes in the two different states.

Work on facultative heterochromatization was initiated in 1921 by Schrader's study of the unusual chromosome constitution of the male mealy bug and was carried further by Hughes-Schrader (*25*) and Schrader. A somewhat similar system, involving chromosome behavior rather than heterochromatization, is present in the dipteran *Sciara*. But most attention

has been focused on the heterochromatization of X chromosomes in mammals.

In the male mealy bug the paternal chromosome set becomes heterochromatic early in development, continues as a genetically inert component, and is finally discarded just before sex-cell formation (*26*). Only the euchromatic, maternal set is transmitted by the fathers to their daughters and sons. In the daughters, the paternal set remains euchromatic, but in the sons it becomes heterochromatic since it was derived directly from the father (Fig. 3). Genetic tests show that the males express and transmit only those genes received from their mothers, and cytochemical tests also show changes. Heterochromatization develops gradually during early embryonic stages (Fig. 4). After the heterochromatic state is established, the heterochromatic set of chromosomes is strikingly evident in the cell nuclei and during early stages of division, but, when the chromosomes are fully condensed at the time of splitting, the two sets can no longer be distinguished (Fig. 4*D*). It is not necessary to assume more than differential condensation to account for the differences in appearance.

In the cat, Barr and Bertram saw a heterochromatic element in the nuclei of nerve cells of females which did not occur in the males; this "sex chromatin" has frequently been called the "Barr body" (*27*). It was later learned that one of the two X chromosomes in mammalian females is heterochromatic whether or not a Barr body is distinguishable in the species; typical cells are shown in Fig. 5. In humans with multiple X chromosomes, only one of the X's is euchromatic; all the remainder become heterochromatic. In the mouse, genes on the sex chromosome showed a mosaic expression; coat color, for example, was a mixture of two types, some patches with the color expected from the maternal genes, the remainder of the patches with that expected from the paternal genes. These observations were integrated by Russell and more completely by Lyon in a now well-known hypothesis which states that the heterochromatic X is genetically inert and that the choice of which X is to remain euchromatic is made at random (*28*).

Heterochromatization of the X occurs in the body of the mouse but not in the cells antecedent to the sex cells, and the two chromosomes are transmitted with equal frequency to the offspring. The function of the heterochromatization is apparently developmental, to equate the number of functional X's in the male (X plus largely inert Y) and the female (X plus heterochromatized X).

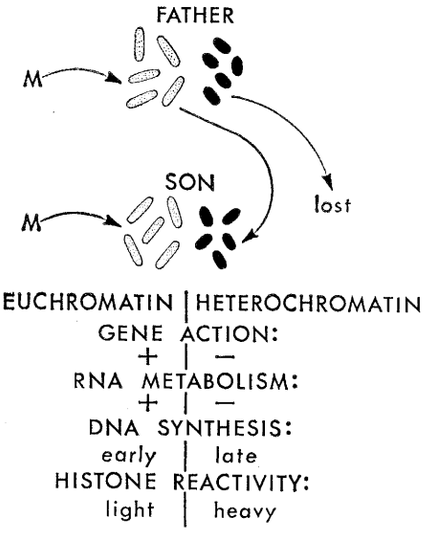

Fig. 3. The mealy bug system. In the males, the maternal set of chromosomes is euchromatic (light) and the paternal is heterochromatic (dark). The heterochromatic set is maintained in the developing body but is discarded at the onset of sperm formation and therefore is not transmitted to the offspring. The male transmits only the euchromatic set which he received from his mother (*M*); on transmission to his sons this set changes from euchromatin to heterochromatin. Concomitant with this change are changes in genetic activity and in nucleic acids and proteins.

Residual effects of the heterochromatized chromosomes have been observed in both the mealy bug (*26*) and the mouse. In the mouse, these effects are apparently attributable to heterochromatization of only part of the X; part remains genetically active (*28*). In the mealy bug they may be due to the return of the paternal set to the euchromatic state in a few key tissues (*29*).

Striking correlations exist between constitutive and facultative heterochromatin. Position effect is demonstrable in the mouse as well as in *Drosophila* (*30*). With autoradiographic techniques, the time of synthesis of new DNA in the cell nucleus can be accurately determined. As Heitz might well have predicted, the heterochromatin duplicates its DNA last in the synthetic period whether it is the constitutive heterochromatin of the X of the grasshopper or the facultative heterochromatin in mammals, mealy bugs (*31*), or marsupials (*32*). All the condensed entities in the cell nuclei of the calf thymus fail to metabolize RNA (*33*), as does also the heterochromatic set of the mealy bug (*34*). The mealy bug thus shows a complete correspondence (Fig. 3) of heterochromatic condensation, genetic inertness, late replication of DNA, failure to metabolize RNA, and a difference in the histone component (*35*).

Constitutive heterochromatin is like euchromatin in its direct response to development. Facultative heterochromatin is also integrated with development, but some extrachromosomal influence must first establish which chromosome is to be heterochromatic, which euchromatic; since both chromosomes are alike genetically, the response to development cannot be simple and direct. In the mealy bug, the paternal chromosomes are probably conditioned before they combine with those of the egg (*26*). In mammals, where the active X is chosen much later in development, it has been suggested that a particle or episome attaches by chance to the one that will remain active (*36*).

In man, a series of clinical syndromes reflects the abnormal status of the sex chromosomes, and the mosiac expression of X-chromosome genes is of both theoretical and practical interest. The most dramatic demonstration yet is that of Linder and Gartler (*37*). Tumors in human females are not mosaics with regard to the expression of genes on X chromosomes; the implication is that these tumors are derived from single cells.

Importance of Doing Nothing

Suggestions have been made before that heterochromatin may serve as a structural material in the chromosome. If we examine the regions where heterochromatin occurs most often, we find that it separates zones of diverse activity. The centromeres are active during cell division, when they guide the new chromosomes to opposite ends of the cells; the majority of the genes are not active during this period. The region organizing the nucleolus produces results visibly different from those of the rest of the genetic material. According to recent data, this region is specifically involved in synthesis of ribosomal RNA (*38*). There seems to be no question, then, that the heterochromatin localized in the vicin-

ity of the centromere and the nucleolar region could have as a function the setting apart of these zones with special activities. Such observations do not, however, prove the point. Chromosome ends are normally stable structures (*2*), and heterochromatin here may provide a simple cap or end piece, thereby excusing active genes from this assignment.

Meiosis is polarized in some instances, so that one of the chromosomes separating from the pair of homologues will not be included in a functional sex cell. Usually there is equal probability of retaining either the maternal or the paternal chromosome, but in some instances one is discarded much more frequently than the other. An extensive series of investigations by Rhoades (*39*) in maize and by Novitski, Sandler, and Lindsley (*40*) and, more recently, Peacock (*41*) in *Drosophila* have shown that heterochromatin is involved in this problem, probably both in contributing to chromosome bulk and more directly in influencing chromosome orientation or centromere potency. Getting into the sex cell is certainly necessary for the evolutionary survival of the chromosome; there is thus no gainsaying the importance of such effects. The effects occur, however, late in the division cycle and may therefore reflect the physical state of the regions adjacent to the centromere rather than influences analogous to typical gene function.

Hereditary factors controlling the behavior of centromeres, such as that of the X chromosome in the dipteran *Sciara* (*42*) or of the supernumerary chromosome of maize (*2*), have been localized in heterochromatin. There is the possibility that these genes are functionally different and that their localization in heterochromatin somehow prevents their interaction with the usual gene. It is also possible that the entity being influenced is affected via a bit of adjacent heterochromatin, and that the system as a whole may have evolved as an extension of the controlling mechanism of or for an originally single heterochromatic entity. McClintock's derivation (*43*) of two-component regulatory systems in maize from others which originally occupied single sites is instructive, whether or not heterochromatin was involved.

The extra chromosomes occurring in some species of plants and animals may vary widely in number. When the number becomes excessive, a generalized debilitation may be induced. These extra, or supernumerary, chromosomes are usually heterochromatic. Extra segments of euchromatin, artificially added by genetic manipulation, are much less readily tolerated and usually produce a specific disturbance in development, characteristic of the segment manipulated. Östergren (*44*) has suggested that these extra chromosomes are parasitic; since their survival depends on the successful completion of the life cycle of the individual carrying them, the less they disturb developmental processes the more often they will be transmitted to the next generation. In some organisms the extra chromosomes are eliminated from those parts of the individual which will not be involved in transmission to the next generation.

The cases of facultative heterochromatization are clearest of all. In the mealy bug, the paternal set of chromosomes is heterochromatized in the male. In certain evolutionary descendants, the paternal set is simply eliminated during early development

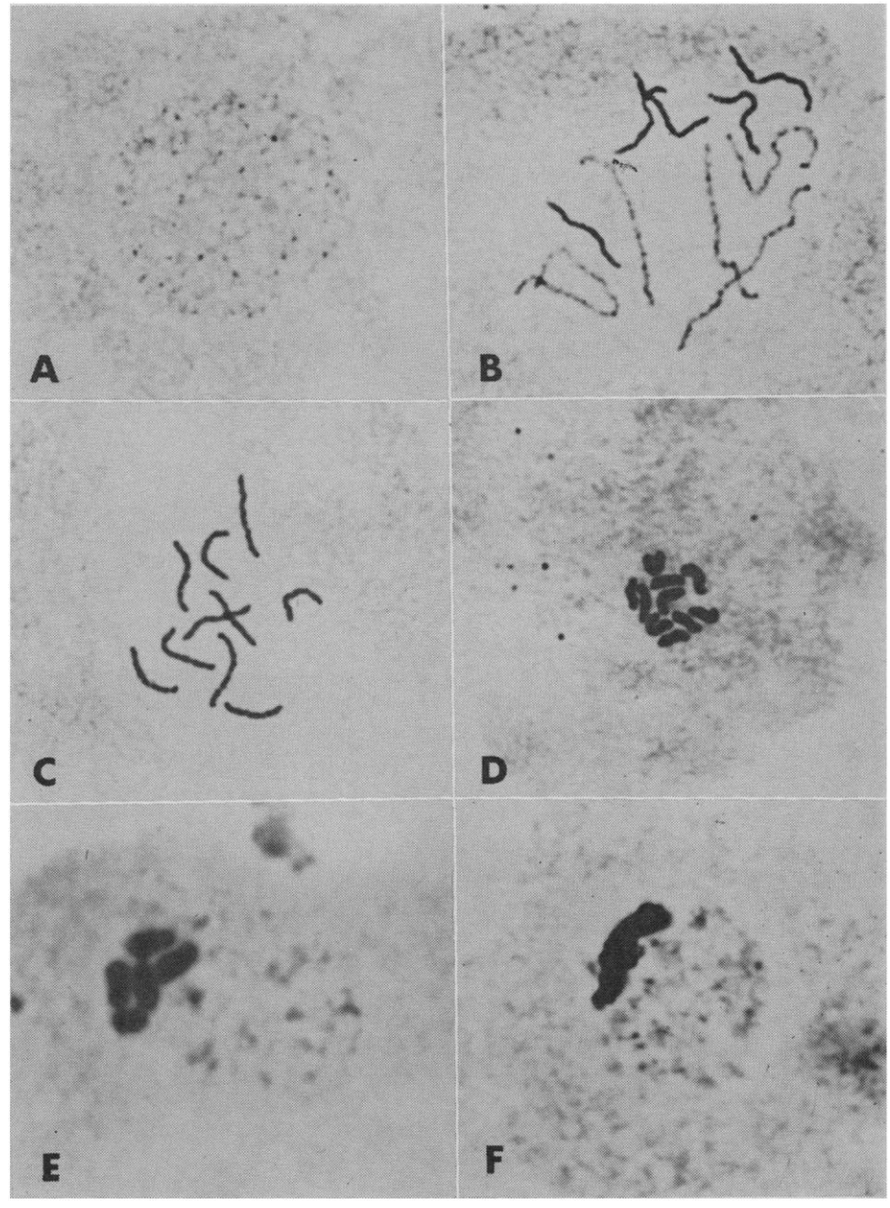

Fig. 4. Heterochromatic chromosomes in a male of the mealy bug. In a young embryo, no heterochromatin appears in the nuclei (*A*). The five chromosomes of the heterochromatic set are, however, strikingly different from the five of the euchromatic set midway through the condensation process (*B*). Further condensation (*C*) obscures this difference, which is quite undetectable when the chromosomes are grouped and ready to divide (*D*). In nuclei of an older embryo, the five heterochromatic chromosomes are sometimes distinct (*E*), sometimes combined in a dense mass (*F*). (*A–D*, × 1600; *E* and *F*, × 2300; photos courtesy M. Sabour)

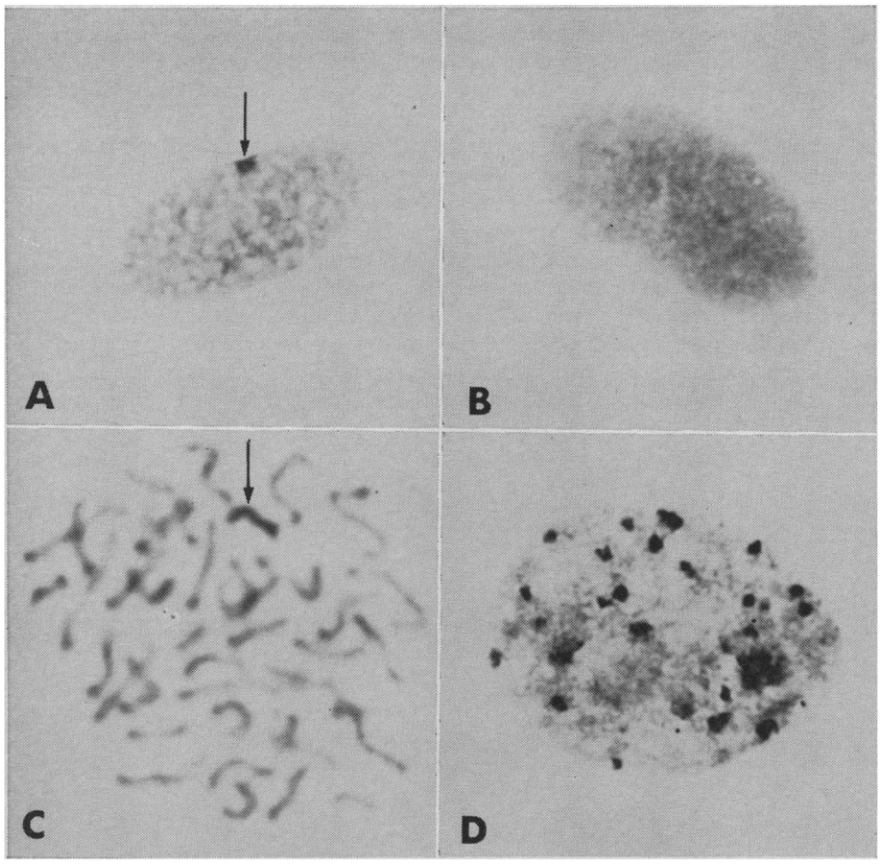

Fig. 5. Heterochromatin in tissue cultures of man (*A*, *B*) and mouse (*C*, *D*). The sex chromatin appears in nuclei of fibroblast cultures taken from the human female (*A*, arrow) but not in those from the male (*B*). In primary cultures from embryonic female mice the heterochromatic X chromosome is identifiable in early phases of the condensation process (*C*, arrow) but not distinguishable among the numerous heterochromatic elements in the nucleus (*D*). (*A* and *B*, about × 2300, courtesy Dr. H. S. Chandra; *C*, × 1800, and *D*, × 1300, courtesy Kathleen K. Church)

and the male progresses further with only the single maternal set, and in collateral forms males develop from unfertilized eggs and thus can have only the maternal set (*45*). There are undoubtedly good reasons, historical, functional, or both, why the heterochromatic set is present in the male mealy bug. The complete absence of the paternal set in related forms indicates its function is minimal, that the organism has not yet quite "learned" how to get rid of it completely.

In both placental mammals and marsupials, one X chromosome in the female is active and euchromatic, the other (or others) is heterochromatic. The heterochromatic X is not present in some species of both mammals (*46*) and marsupials (*47*), and the female progresses, except in the formation of the sex cells, with only the single active X.

In the longer reaches of evolutionary history, a similar fate befalls the constitutively heterochromatic Y. Its functions are gradually lessened until it disappears. The sex chromosome constitution then becomes XX in the female and XO in the male, the "O" indicating no Y chromosome.

Many developmental biologists believe that most genes are in general nonfunctional during early embryonic stages. Both facultative and constitutive heterochromatin may fail to appear, or may appear gradually, during these early stages. Beermann (*48*) has recently pointed out that, in general, chromosomes in young embryos are morphologically different from those in older, functioning tissues. Such developmental changes may soon be correlated with the onset of RNA metabolism during embryogeny (*49*).

In a study too little appreciated in recent genetic thinking, Clausen and Cameron (*50*) proved conclusively that unnecessary genes lose their functional abilities in relatively brief evolutionary periods. If we apply this concept to chromosome segments inactivated by heterochromatization for a sufficient period, we must expect that here also functional abilities will be lost. Thus later reversions to the euchromatic state would free the genes for action, but the genes would no longer be able to act.

In spite of the ambiguity of some of these cases, the overall import is quite clear: the evolutionary and developmental significance of the heterochromatic state lies in its capacity for shutting off normal gene function. Heterochromatic segments may in part be active. Certain genes might survive the change of state; new mutants might arise capable of functioning in the heterochromatic state; bits of euchromatin may be intercalated in the heterochromatin. These departures from complete inertness will be detectable by cytogenetic techniques. What we do not have for most cases of constitutive heterochromatin are techniques for testing the significance or function of the relative inertness itself. At present, we can only infer a significance, of some sort, from the ubiquity of certain localizations such as that of the centric heterochromatin.

Conclusions

The subject of heterochromatin is one of the most difficult and diffuse in modern biology. We are often dealing with information of a more-or-less type which is impossible to evaluate precisely or to score quantitatively. We have a variable cytological picture which has yet to be followed completely through the development of any organism. We have fairly good genetic evidence that constitutive heterochromatin, at least as it exists in typical, non-giant cells, is the home of relatively few genes and that facultative heterochromatization shuts off the genes in the affected areas. But some genetic effects are traceable to both types of heterochromatin, or to genes adapted to survive in heterochromatin.

We do not have the necessary information to fill in much further detail. Such basic facts as the number of elemental strands per chromosome still elude us. The systems controlling gene action in higher organisms probably involve highly complex mechanisms necessary for developmental integration. We may take our pick, at present, of a variety of substances any of which may be involved in these control mechanisms. The overall regulation

which all the chromosomes undergo when they condense during a division cycle is also a prime area of ignorance.

There is no question that these and many similar problems must be solved before we begin to understand genetic systems in higher organisms. The major virtures of heterochromatin are two: large chromosome segments are frequently involved in the heterochromatic state, and we may follow them visually both through the processes of cell mechanics and development and through their evolutionary modification. In spite of the ambiguities, the study of heterochromatin remains a worthwhile task.

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NEWS AND COMMENT

R & D Funds Show Effects of a Tough Budget Year

The proposed federal budget for the 1967 fiscal year is the first budget since the middle 1950's, when federal science acquired the general structure familiar today, which has not carried a request for an increase in total funds for research and development.

In the budget which the President sent to Congress on Monday, expenditures for R&D activities would be $15.939 billion, some $22 million less than the estimated $15.961 that will be spent in the current fiscal year, which ends 30 June.

This downturn in what for about a decade has been a steadily ascending curve directly reflects the major rationale of the new budget: to provide funds to support a military buildup in Southeast Asia without underfinancing new education and welfare programs enacted during the past two sessions of Congress under the banner of the Great Society.

The impact on the science budget is created less by cutbacks than by the lack of increases which the scientific community has grown accustomed to expect. Shifts of funds within the budget have, in the case of some programs and some functions, resulted in fairly substantial increases. Funds for basic research, for example, would rise under the new budget. But there is a built-in "creep" of costs in research, and it is expected that this will be a tight year, especially for new research and training grants.

The largest single reduction to affect any science agency is a cut of $300 million in funds requested for the National Aeronautics and Space Administration. The new budget asks for $5.3 billion for next year, compared with $5.6 billion in the current fiscal year. Up to now, the NASA budget has been bigger each year since the agency was established in 1958. Reductions are possible this year, say administration officials, because a number of space-agency programs are moving from the expensive development stage to the less costly operational phase. Financially most significant is the fact that costly facilities at the Houston manned space-flight center and at Cape Kennedy are completed or nearly so. This winding up of major construction projects accounts for some $200 million of the reduction in costs which have hitherto been charged to the R&D budget.

Cuts in construction are, as a matter of fact, a cost-reducing factor which affects not only the science budget but the whole federal budget for the coming fiscal year. In the science budget, obligational authority for construction (for funds which would be obligated but not necessarily spent in the 1967 fiscal year) would be reduced from $849.3 million in the current year to $617.4 in fiscal 1967 (see Table 4).

This cutback in construction has two major purposes. In addition to making more funds available for military expenditures and Great Society programs, the cuts would take federal money out