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# Discovery of Controlling Elements: A Mcclintock's View

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**Introduction:**

**Mcclintock's Journey to The Sleeping Beauty**

The unit of inheritance What Mendel referred to as 'factor' was renamed as gene by Wilhelm Johannsen in 1909. Between 1907 and 1919, the geneticist Thomas Hunt Morgan and his colleagues conducted a series of experiments on the fruit fly (*Drosophila melanogaster*), and observed a linkage between white eye color and gender of the flies and concluded the location of gene responsible for white eye color on the X chromosome, Morgan and co-workers showed that each gene for a particular trait was located at a specific spot, i.e., a locus, on a chromosome in all individuals of a species (Gellman, 2020). Experiments by Morgan established the fact that each gene is situated over a specific site in a genome, until she discovered transposition of *Ds*and *Ac* loci in maize, what are known today as transposable elements; McClintock called 'controlling elements (Comfort, 2001). In 1950 Barbara McClintock's paper 'The Origin and Behaviour of Mutable Loci in Maize', and her famous presentation at the Cold Spring Harbour Symposium in 1951 constituted an important chapter in the field of molecular genetics, recognized by the world 33 years later when awarded by Nobel prize in 1983.

At Cornell's College of Agriculture, McClintock developed various staining techniques in maize for easy observation of maize chromosomes, with time McClintock mastered these techniques at such an extent that she was able to differentiate between the 10 chromosomes of the maize, By 1932McClintock published nine articles on maize chromosomes, including studies of the centromere and the nucleolus, and a 1931 PNAS article in which she demonstrated genetic crossing-over at the chromosomal level and showed that genetic recombination involved the physical exchange of chromosome segments (Ravindran,2012). Between 1935 and 1941, at the University of Missouri, Columbia, while her studies on X-ray-irradiated maize to map genes, she observed that in one of her strains, spontaneous breakage,

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and fusion of chromosome arms, which she described as 'breakage-fusion bridge' cycle was very frequent (Krishnaswamy, 2007).In 1942 she was appointed as a researcher at the Carnegie Institution of Washington's (now Carnegie institute for science) Department of Genetics at Cold Spring Harbor and continued her research on the strains which showed 'breakage-fusion bridge' cycle where she discovered a pair of genetic loci in maize (*Ds/As*) at short arm of chromosome 9 that seemed to trigger spontaneous and reversible mutations in ordinary, stable alleles. She tried to map these loci but by early 1948, difficulties in mapping the loci led her to conclude that they were not loci – sites on the chromosomes – but chromosomal elements that moved from place to place (Comfort,2001). In 1950s Peterson, identified the Enhancer-Inhibitor (*En/I*) element Almost simultaneously, McClintock identified the Suppressor-mutator (*Spm/dSpm*) element. Later it was shown that these two systems, *En/I* and *Spm/dSpm*, are identical (Krishnaswamy, 2007). Previously in 1936Marcus Rhoades observed frequent mutations in Mexican Black corn, giving it a dotted phenotype (anthocyanin pigmentation in the aleurone of the endosperm), according to Rhoades the dotted phenotype of *al* gene was controlled by the Dotted (*Dt*) gene. McClintock proposed that *Dt* was a controlling element analogous to the *Ac/Ds* system, and Nuffer established that the dotted phenotype observed in Rhoades' plants was caused by the activity of the *Dt* transposable element (Krishnaswamy, 2007). By the mid-1960s advanced discoveries in molecular genetics provided tools for gathering evidences in the support of transposons.

In 1967 James Shapiro, then at the Postgraduate Medical School of London, and Sankhar Adhya, at the University of Wisconsin, published their discovery of insertion sequence (IS) elements in bacteria (Comfort,2001) i.e., transposons after which similar elements were found in various other organisms and by mid-1970s transposons were the next big thing in the field of genetics In June 1976, at a Cold Spring Harbor meeting organized by Shapiro, Adhya and Ahmad Bukhari, transposable genetic elements or 'transposons' were defined simply as pieces of DNA that can move from one place to another (Comfort,2001). In 1976, McClintock got nominated for the Nobel Prize by Judson van Wyk and rest we know is history.

**Breakage-Fusion-Bridge Cycle**

McClintock major work was conducted on maize, radiation treatment on maize by McClintock gave birth to some cultures with broken ended chromosome 9, which was further explained by McClintock in her paper '*The stability of broken ends of chromosomes in Zea*

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*mays*' published in 1940 and '*The fusion of broken ends of chromosomes following nuclear fusion*' published in 1942.McClintock majorly conducted cytological studies to understand chromosome 9 with broken ends, however for such studies radiation treated Chromosomes with deficiencies cannot be used as they are not transmissible through the gametes so to overcome this, the broken ends were generated by crossing over in plants carrying a normal chromosome 9 and a homologue with an inverted duplication at the end of the short arm. Due to crossing over in the inverted duplication region a bridge is formed at I meiotic anaphase which break at a site and now each nucleus formed in first meiotic telophase owns a broken ended chromosome following which in II meiotic prophase the sister chromatids fuse with each other as both have sticky ends (broken ends), in II meiotic anaphase they move to opposite poles of cell forming a bridge configuration followed by breakage because of tension producing gametophytes (pollen grains in male embryo sac in females) with broken ended chromosome 9. This cycle of breakage-fusion-bridge (BFB) continues in successive mitotic divisions while production of gametes in gametophytes. Therefore, all the nuclei of the fully developed male or female gametophyte will possess one chromosome with a single broken end (McClintock, 1942), that is gametes with broken ended chromosome 9. McClintock thus had a method of producing large numbers of gametes which carried a chromosome 9 with a broken end, and which was not deficient for any of its genes (Jones, 2005), McClintock used them in various combination of crosses to study cytology of chromosome 9 in meiotic and mitotic divisions occurring in embryos and endosperm of maize kernels

while studying plants whose one homologue has broken, telomere-less chromosome 9 she gave a term Chromatid breakage-fusion-bridge cycle, in such plants chromosome with broken end during the miotic prophase replicates to form two sister chromatids and obviously both halves have broken ends or to say sticky ends which fuses with each other obtaining a dicentric chromatid which is the equivalent of two chromosomes 9 attached at the ends (McClintock, 1942), following which during mitotic anaphase spindle fibres gets attached to two centromeres of dicentric chromatid and pulls them to periphery of the cell causing to form a chromatin bridge, tension on the bridge breaks it randomly on any site and again we end up with chromosomes with sticky ends which either heal themselves or continues the cycle, this process was named as chromatid breakage-fusion- bridge cycle and it was only observed in gametophytes and endosperm (Jones, 2005)

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Similarly, her cytological studies on chromosomes of plant obtained from crosses where both parents contain a chromosome 9 with broken end (will provide progenies were both homologue of chromosome 9 have broken ends), she gave the term Chromosome breakage-fusion-bridge cycle. After the fertilization of gametes, she obtained a zygote with two homologue of broken ended chromosome 9 and a triploid endosperm cell with three homologue of broken ended chromosome 9, chromosomes present in endosperm showed the previously observed chromatid type BFB cycle but in the embryonic cells following fertilization we get a diploid cell with two homologue both with broken ends which replicates in mitotic prophase, fuses and form a bridge in mitotic anaphase and after breakage broken ended chromosome pass on to two sister nuclei in miotic telophase, both the sister nuclei again owns two homologues of broken ended chromosome 9 which this time fuses together before there replication in coming prophase stopping the chromatid type BFB cycle this variation was named as Chromosome breakage-fusion-bridge cycle by McClintock. On the basis of her experimental studies McClintock concluded the following points in her paper 'The stability of broken ends of chromosomes in zea mays'

1. A chromosome with sticky ends when reaches the primary endospermic nucleus through either the male or the female gametophyte during the fertilization, the chromatid type BFB cycle will take place in developing endosperm that is cycle continues during successive mitotic divisions.
2. Similarly, if broken ended chromosome 9 reaches the zygote threw male or female gametes, chromosome type BFB cycle will take place in developing embryo ceasing further repetition by healing the broken ends.
3. The BFB cycle is confined to the gametophytic and endosperm tissues of the generation immediately following the initial break in the chromosome (McClintock, 1940) as the chromosome type BFB cycle takes place in embryo, stopping it to be inherited to its progeny
4. When healed broken chromosomes are passed on from one generation to another, the succeeding generation don't show any signs of BFB cycle.

Variegation In Maize Kernels

In 1944 while working on the genetic composition of the short arm of 9$^{th}$ chromosome of maize she witnessed that the progeny of 450 self-pollinated plants of maize whose short arm

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of 9th chromosome was subjected to structural modification due to chromosome type breakage-fusion-bridge cycle, showed the variegation or to say a sudden burst of unstable mutations, these new series of events raised questions that If the chromosome type BFB cycle ceases in the developing embryo then how these variegation which arise because of BFB cycle are inherited to the progenies of maize plant. Terms like mutable genes, unstable genes, variegation, mosaicism, mutable loci or "position-effect" were associated with this previously observed phenomenon, such phenomenon were observed in various organisms but McClintock believed that the mechanism underlying the phenomenon is basically the same in all organisms. Pachytene analysis of these progenies also revealed the significant amount of chromosomal rearrangement due to deficiency, duplication, breaks, translocations, inversions and knob fusions in other chromosomes apart from chromosome 9, giving rise to 32 new stable mutants. McClintock knew from her accumulated knowledge of chromosome-breakage and endosperm variegation that they were the result of breakage events and that the chromatid BFB cycle was going on. The breakage evidently differed from anything she had seen before because it was "controlled" and was being initiated at specific times in the development of the endosperm (Jones, 2005). Following which McClintock started digging around the phenomenon by studying the maize mutants showing variegation.

McClintock cleverly understood the direct impact of BFB cycle on origination of variegation in seeds, she stated that 'If a broken chromosome continued the breakage-fusion-bridge cycle in successive nuclear divisions, its presence should be made evident by genetic variegation in endosperm and plant tissues' but only when the broken ended chromosome contains the dominant alleles and normal homologue contains recessive alleles, as the BFB cycle causes deletion of dominant alleles from one nuclei and duplicates it in sister chromatid the cells containing dominant allele show their presence this repeated cycle causes each cell in developing endosperm with different sets of dominant alleles or no dominant allele, resulting in maize seeds with variegation it goes unsaid that this phenomena is restricted to present generation and not inherited in upcoming generations.

**Mcclintock's View of Controlling Elements**

McClintock focused her further studies on the coloured patterns and marker genes associated with them. short arm of chromosome 9 consists of marker genes $Yg^2$(*Yg*-normal green plant, *yg*-yellow-green plant), *C*(*C*-coloured aleurone, *c*-colourless aleurone), *Sh*(*Sh*-

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normal development of the endosperm, *sh*-shrunken endosperm), and *Wx*(*Wx*-normal starch staining blue with iodine, *wx*-waxy starch staining red with iodine)(McClintock, 1940), but no sticky ends, further studies showed Variegation but there were no "sticky" ends present to initiate the BFB cycle. The kernels were colorless with spots of bronze pigmentation due to expression of the recessive *bz* allele. When the kernels were scratched and stained the colorless regions were found to have the *Sh* and *Wx* phenotypes and the colored areas to have the phenotype determined by the recessive alleles *sh* and *wx*. In other words, the colored sectors were lacking in all four of the dominant alleles for characters of the endosperm. Pachytene analysis of chromosomes revealed a breakage in one of the two homologues of the short arm of chromosome 9 in some of the plants always at the same site, which was named as *dissociation* or *Ds* locus, while on the other hand some kernels showed no variegation but they had *Ds* breakage. Males carrying *DsDsCC* locus on both homologues were crossed with females carrying *dsdscc* (without breakage and colorless) and all of the progeny were expected to be heterozygous with variegated kernels due to loss of the *C* allele, but half of the progenies turned out to be normal. This made McClintock to conclude that there must be some other factor presence of which is essential for breakage to occur at *Ds* locus. McClintock named the factor as *activator* or *Ac* element, which inherited independently of *Ds*.

**Conclusion**

With time various studies helped in understanding the mechanism of working and different other aspects of this phenomenon, McClintock's work certainly was ahead of her time showing how less is known about the field of genetics and allied sciences, today various systems of transposable elements are known and are being used in the field of genetic engineering to introduce a piece of foreign DNA or in field of plant breeding for elimination of genetic drag etc.

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