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An Attempt at Identifying the Position of Genes on the Chromosomes of Maize using X-Ray Induced Chromosome Deficiencies

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Abstract

Once McClintock established that in maize specific genes were physically localized on a particular chromosome, she set out to establish the physical location of genes on a chromosome. To do this, she performed cytological studies on Lewis Stadler's X-ray mutated corn plants. The X-rays caused a portion of a chromosome to be eliminated that resulted in the production of a rod-shaped chromosome with a deficiency and a ring-shaped chromosome that contained the genes deleted from the rod-shaped chromosome. As long as the ring-shaped chromosome contained a centromere it could be transmitted from cell to cell. If the ring chromosome were small, one could assume that the genes carried on it came from a region near the centromere. The smaller the ring chromosome, the better was this assumption. If and when the ring chromosome was lost, the plant would become variegated and from the nature of the variegation, one could assign a position to the gene that coded the character that is variegated to a region close to the centromere.

Once Barbara McClintock (1929) cytologically identified ten chromosomes of maize and assigned six of the ten linkage groups to the chromosomes (McClintock & Hill, 1931; Kass and Bonneuil 2004: 106-107), she could then begin a study to localize genes on the chromosomes. In order to accomplish this goal, McClintock (1931, Birchler, this volume; McClintock, 1932) joined with Lewis Stadler who was able to use X-rays to induce deficiencies (deletions) in chromosomes. Stadler suggested the initial problem to McClintock and furnished her with the mutated maize plants (McClintock, 1931). Stadler became interested in genetics after reading T. H Morgan's (1919) book, *The Physical Basis of Heredity*, and, perhaps after he realized that chromosomes were indeed real physical entities, he sought out a physical method that would be penetrating enough and small enough to perturb discrete regions of a chromosome mechanically. Stadler was among the pioneers who discovered that gamma rays and X-rays had the ability to cause alterations in the chromosomes of plants and animals (Blakeslee, 1927; Gager and Blakeslee, 1927; Muller, 1927; Curtis, 1928; Stadler, 1928a, b, 1930a, b, 1932, 1936; Anderson, 1936; Rhoades, 1957, 1984). The collaboration between Stadler and McClintock resulted in a paper entitled, "A correlation of ring-shaped chromosomes with variegation in *Zea mays*."

Stadler (1928a, 1931a) had developed a technique in which he X-ray irradiated maize pollen marked with dominant allelomorphs (alleles) for endosperm characters in order to induce chromosome deficiencies. He would then be able to infer the relationship between a physical segment of the chromosome and the trait it caused in a given cell. To find such a relationship, Stadler pollinated a female parent that was marked with recessive allelomorphs for endosperm characters with the X-ray irradiated pollen that was marked with the dominant allelomorphs. If a dominant allelomorph were lost as a result of an X-ray induced loss of a segment of chromosome, the resulting endosperm cell would express the recessive character. By marking the chromosomes with two or more linked allelomorphs, Stadler could get an idea of the spatial extent of the chromosome loss. The endosperm was particularly useful for these genetic studies since, as a result of double fertilization, its characteristics depend on both the maternal genotype and the paternal genotype (xenia; East, 1913) and the endosperm characters were visible a short time after pollination and long before the seed germinates.

McClintock spent the first part of her National Research Council (NRC) Fellowship (1931-1932) at Missouri, working on the cytology of Stadlers' plants. McClintock (1931) used plants from Stadler's X-ray mutant lines, which had lost dominant alleles of the genes *Lg*, *A*, *Pl* and *R*. By observing cytological deficiencies correlated with genic loss from pollen or embryo treated plants, McClintock located the genes for *liguleless* (*lg*, ligule absent) on the *B-lg* chromosome [chromosome 2], *anthocyaninless* (*a*, green plant color absent) on the *A-d1-cr* chromosome [chromosome 3], loss of *purple* color (*pl*) on the *Pl-Y*, Satellite chromosome [chromosome 6], and loss of *red* color (r) on the *R-g* chromosome [chromosome 10].

During her initial investigations, McClintock (1931, p. 22) first recognized a ring chromosome with its insertion region [centromere] in corn, which she hypothesized may have been derived from a deleted rod shaped chromosome. Such a chromosome was first described by Navashin in 1930. McClintock believed that the variegated plants that Stadler had observed, may have been due to loss of ring chromosomes carrying dominant alleles. She returned to Missouri during her second NRC fellowship (1932-1933) to investigate these variegated plants.

Stadler used pollen that possessed the dominant allelomorphs *Lg*, *B* and V_4, which encode for *ligules, sun-red* plant color, and *nonvirescent* seedlings, respectively (Fraser, 1939). The pollen was placed on the stigma of a plant that possessed the recessive allelomorphs *lg*, *b* and v_4. As long as the pollen had not been irradiated with X-rays, the chromosomes should be rod shaped and transmitted from cell to cell in the normal way so that all the cells in the plant would be genetically identical and all should show the dominant phenotypes without variegation.

However, when Stadler used *Lg*, *B* and V_4 pollen that had been irradiated with X-rays to pollinate a plant that contained the recessive alleles, *lg*, *b* and v_4, the progeny were not heterozygous with a dominant phenotype but were *ligule-less* (*lg*), probably *virescent* (v_4) and consisted of stalks and leaves with *sun-red* and *green* stripes. When McClintock (1932) looked at the microsporocytes in mid-prophase from the anthers, she noticed that a ring-shaped chromosome synapsed with the normal rod-shaped chromosome *B-lg* [chromosome 2 (Kass and Chomet, 2009)]. By studying the chromosomes during synapsis, McClintock could see that the ring-shaped chromosome was missing both ends of the chromosome—one that contained the *Lg* allele and one that contained the V_4 allele. This would explain the observation that the progeny were all *ligule-less* and probably *virescent*. The free ends of the middle of the chromosome, which is the part that contained the *B* gene, fused to form the ring. McClintock observed that in many microsporocytes, the ring-shaped chromosome was obviously diminished. Assuming that the presence of a ring-shaped chromosome was cytological evidence for a gene deficiency, McClintock surmised that the cells that made up the *sun-red* stripes must have a ring-shaped chromosome that contained the *B* allelomorph while the cells that made up the *green* stripes must have had a ring-shaped chromosome that lost the segment that contained the *B* allelomorph. However, this was just an untested hypothesis since McClintock only correlated an increase in *sun-red-green* variegation with the presence of X-ray induced ring-shaped chromosomes.

X-ray irradiation could produce a deficiency in any chromosome and result in a ring-shaped chromosome. The *Pl* allelomorph, which coded for *purple* color in the leaves and stalks, resided on the satellited chromosome [chromosome 6 (Kass & Chomet, 2009)]. When Stadler pollinated a plant that contained the recessive allelomorph, *pl* with *Pl* pollen that had been irradiated with X-rays, the progeny were not heterozygous with a dominant phenotype but consisted of stalks and leaves with *sun-red* and *purple* stripes. The *sun-red* stripes indicated that the dominant *Pl* allelomorph was no longer expressed. McClintock (1932) found patches of cells that had very small ring-shaped chromosomes. Presumably each of the *sun-red* cells had a small ring-shaped chromosome that lacked the segment that contained the *Pl* allelomorph. Again, this was just an untested hypothesis since McClintock only correlated an increase in *purple-sun-red* variegation with the presence of X-ray induced ring-shaped chromosomes.

McClintock (1932) studied the Bm_1 and the bm_1 allelomorphs that resulted in *colorless* or *brown* cell walls, respectively. The *brown*-walled or *colorless* phenotypes were visible in cross sections of the stem or as *brown* veins (vs. colorless veins) in the stalk and leaves. When Stadler used Bm_1 pollen that had been irradiated with X-rays to pollinate a plant that contained the recessive allele, *bm1*, the progeny were not heterozygous with a dominant phenotype but consisted of leaves and leaf sheaths with a variegation that resulted in patches of *brown* and *colorless* veins. The *brown* veins (*bm1*) indicating that the dominant (clear veined) Bm_1 allele was no longer expressed.

Cytological examination of these plants revealed that during meiosis ten bivalents and a ring-shaped chromosome were present. In these plants, which had 21 chromosomes, an arm of the Bm_1 chromosome [chromosome 5 (McClintock, 1938)] was shorter than in the unirradiated plant and the size of the deletion seemed to be comparable to the size of the ring-shaped chromosome. In one plant, the arm of the maternal *Bm1* chromosome buckled when it synapsed with the paternal chromosome. In another plant, which had a tiny ring-shaped chromosome comprised of not more than several chromomeres; synapsis between the maternal and paternal rod-shaped *Bm1* chromosomes showed no buckling. Given that both the rod-shaped chromosome that gave rise to the tiny ring-shaped chromosome and the tiny ring-shaped chromosome itself had insertion regions or centromeres, McClintock hypothesized that the Bm_1 gene must be localized close to the insertion region. However, based on other evidence not presented, McClintock was unable to make any decisive statement concerning the position of the Bm_1 deficiency. In follow-up work, McClintock (1938) presented cytological evidence that confirmed her hypothesis on the localization of the Bm_1 gene near the centromere.

McClintock observed that throughout development, the ring-shaped Bm_1 chromosome tended to get smaller and smaller and eventually got lost. McClintock proposed that the loss of the Bm_1 allelomorph was a result of the degeneration or complete loss of the ring-shaped Bm_1 chromosome was the likely cause of the variegation in the brown-colorless cell wall chimera [i.e., variegation]. While the ring-shaped chromosome typically decreased in size or disappeared, McClintock also found that they could increase in both size and number. Although McClintock did not yet know the mechanism for the decrease or increase in size of the ring chromosomes in her corn plants, she hypothesized that rings might become interlocked. Movement of insertion regions [centromeres] toward opposite poles, she suggested, would cause breaks in the ring chromosomes causing changes in their size and genic makeup. To support her hypothesis, she again cited the studies of Navashin (1930), who had presented "figures of two interlocking rings in a somatic metaphase in *Crepis* [hawksbeard, Asteraceae]."

It is a commonplace that rod-shaped chromosomes are typically transmitted from cell to cell so that each cell has the same genetic complement. By contrast, ring-shaped chromosomes are not as persistent in that all or part of the ring-shaped chromosome is lost during mitosis. Consequently, the daughter cells of plants with ring-shaped chromosomes are not genetically identical and the plant develops a variegated, chimeric or mosaic phenotype. Since the ring-shaped chromosomes can also form spontaneously (McClintock, 1932), McClintock knew from Stadler's (1931a, b) investigations, that it is possible that plants, which had not been subjected to X-rays, may also be genetic chimeras. Having the knowledge that not all cells in a single maize plant had the same genetic constitution, McClintock may have developed a mind that was prepared to find transposable elements. As Joseph Henry said in his presidential address to the Philosophical Society of Washington on November 24, 1877, "*The seeds of great discoveries are constantly floating around us, but they only take root in minds well-prepared to receive them*" (Bauer, 1908).

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