Материалы докладов на VI научной школе молодых ученых

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HOW BARBARA MCCLINTOCK DISCOVERED TRANSPOSABLE ELEMENTS IN MAIZE

❀ **The paper describes the early part of Barbara McClintock's work on DNA transposons in maize, in which she discovered the Ac-Ds family of mobile "controlling elements". An account is first given of the cytology of the system that was used to generate intact chromosomes having "sticky" (broken) ends. Cytogenetical aspects of the chromatid and chromosome breakage-fusion-bridge cycles, deriving from breakage, are then described, which leads on to the way in which variegation in phenotypes of the maize kernels could be "read" in terms of chromosome breakage. The "genetic earthquake" event of 1944, triggered by introducing broken chromosomes into a zygote from both parents, lead to the discovery of Ds and Ac. Finding mobility of Ds from one chromosomal location to another was pure serendipity: the transposition showed itself while experiments were being undertaken to accurately map Ds. A similar chance observation revealed transposition of Ac as well, and then the relationship between the two elements was elucidated in terms of their autonomous and non-autonomous nature.**

❀ **Key words:** DNA transposons; McClintock; breakage-fusion-bridge cycle; Ac-Ds elements; maize cytogenetics.

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INTRODUCTION

McClintock discovered DNA transposons while working with *Zea maize* in the 1940s, through cytogenetical analysis of the phenotypes of maize kernels, initially involving the *Ac-Ds* family of mobile elements. This paper reconstructs the story of the early part of her work and traces the steps by which she unravelled the genetics of the *Ac-Ds* family of "controlling elements". Her main publication came from her lecture at he Cold Spring Harbor Symposium in 1951 (McClintock, 1951). There are later reviews dealing with the *Ac-Ds* system, as well as with other independent transposons in maize, and also with McClintock's ideas on the way in which mobile elements may lead to genome reorganisation (McClintock, 1956, 1965, 1978). The early results of the *Ac-Ds* story appear in the annual volumes of the Carnegie Institution of Washington Yearbook, from 1942 onwards (Jones, 2005), and are not cited individually in this text. Earlier Investigations on chromosome breakage, which precede the controlling element reports, can be found in McClintock 1939, 1941, 1942. Reviews on the *Ac-Ds* system by other authors include Fincham and Sastry (1974) and Fedoroff (1983). An account of the molecular cloning of the *Ac* and *Ds* elements is given in Fedoroff 1984.

STRUCTURE OF MAIZE KERNELS

The maize ear contains many individual kernels which are the products of fertilisation, and which are numerous enough to allow conclusions about genetics to made from single ears. The embryo and endosperm are both products of fertilisation, from a cross or self pollination. The pericarp is derived from the ovary wall and is maternal tissue, showing the characters of the maternal parent. A number of genes are known which determine traits of the kernel. They mainly involve pigmentation of the aleurone layer and the pericarp, and also the quality of the starch in the endosperm. Many of these phenotypes are also non-essential for viability. The pericarp resembles the tissues of the plant in the way that its cells are in lineages, so mutations produce streaks of colour, whereas instabilities of the endosperm appear as spots, because the cells are a disorganised structure. Mutations arising early in endosperm formation will produce large sectors of altered phenotype, and those happening later will give smaller patches.

CHROMOSOME BREAKAGE

In the late 1930s McClintock studied the behaviour of chromosomes having a single broken end, in order to determine the behaviour of broken ends in mitosis (McClintock, 1939). The work largely concerned breakages in the short arm of chromosome 9 (9S), which carried several useful markers affecting pigmentation of the aleurone, quality of the endosperm starch and characters of the plant phenotype. Chromosomes with deficiencies are not readily transmissible through

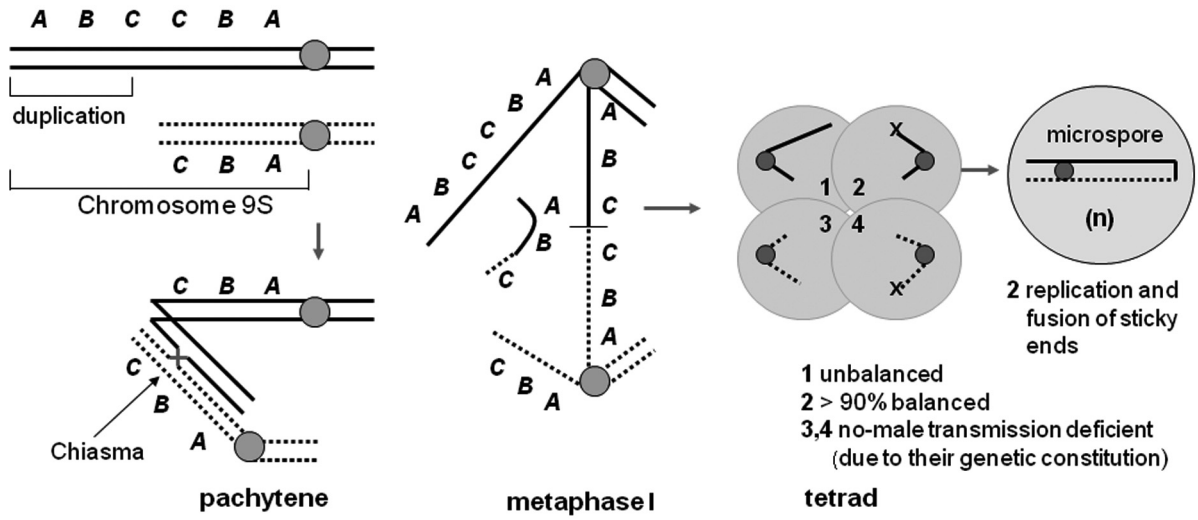

Fig. 1. The generation of "sticky" ends in the short arm of chromosome 9 by use of a duplication and crossing over to produce a broken end without any loss of genetic material (x = sticky ends)

the gametes (especially on the male side), so a way of generating broken ends of 9S without causing any terminal deletions was devised. These "sticky" ends were generated by crossing over in structural heterozygotes carrying a normal chromosome 9 and a homologue with an inverted duplication at the end of the short arm. Following crossing over in the region spanned by the duplication a bridge is formed at AI, which may then break at various places to produce the sticky ends in a high proportion of the gametes (Fig. 1, Fig. 2). Cytological observations indicated that 18 % of microspores carried the broken 9, and that only 3.6 % were recovered from pollen of these plants, presumably because of imbalance. Pollen with the duplicate 9 does not compete well with pollen carrying normal 9, and the pollen that mainly functions carries the normal 9. However, if in such plants the normal 9 is replaced by a chromosome 9 with a non-male transmissible deficiency then only the pollen with a duplication, or a broken 9, will function. Broken 9 is less unbalanced than duplicate 9, so competitive pollen tube growth favours broken 9. Broken 9 thus functions in 75–90 % of cases (based on tests of 30,000 pollen grains). McClintock thus had a method of producing large numbers of gametes which carried a chromosome 9 with a "sticky" end, and which was not deficient for any of its genes. She could introduce these broken chromosomes into crosses and then see how they behaved in mitotic divisions of the embryo and endosperm.

CHROMATID BREAKAGE-FUSION-BRIDGE CYCLE

One of the first results of the breakage studies was that the broken chromosomes behaved differently in the embryo and endosperm during development. In the embryo the broken homologue underwent "healing" of its "sticky" end, almost immediately after fertilisation, and thereafter mitosis was normal. In the triploid endosperm this healing did not occur. The two unbroken chromosomes 9, contributed by the female parent, underwent the normal mitotic cycle, but the broken 9 from the male did not behave in this way. At the first replication following fertilisation the "sticky" ends of the two sister chromatids fused together again, so that separation at anaphase was impossible.

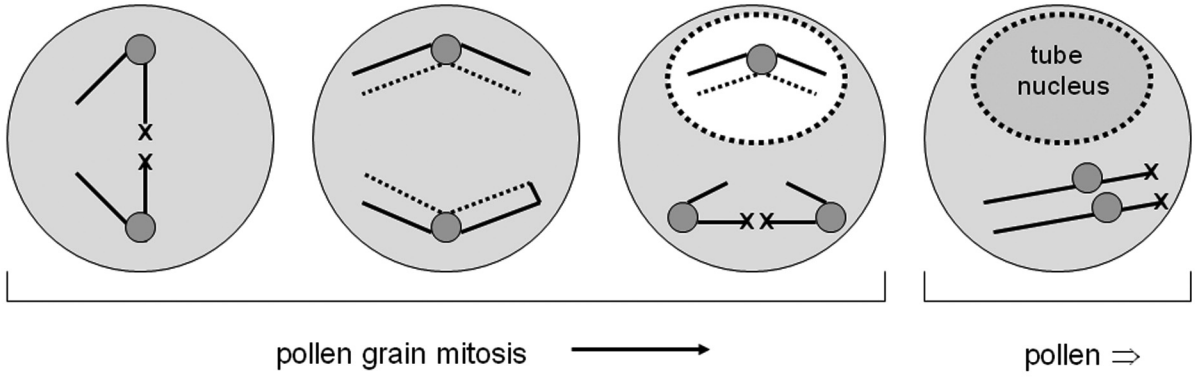

Fig. 2. Pollen grain mitosis and the formation of sticky ends in chromosome 9 of the sperm nuclei (x = sticky ends)

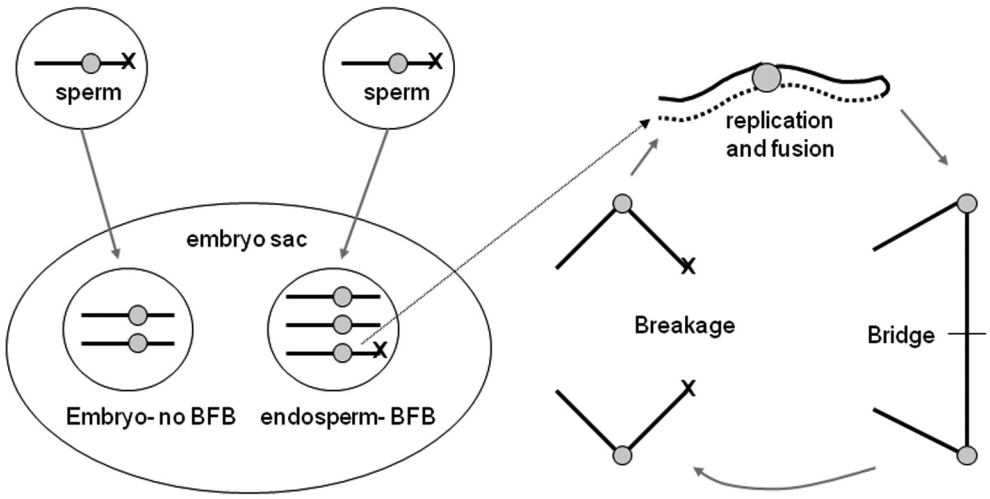

Fig. 3. The chromatid breakage-fusion-bridge (BFB) cycle in the endosperm (x = sticky ends)

A bridge was formed again joining the two centromeres (Fig. 3). During anaphase the pull of the centromeres stretched the bridge and caused it to break, so that each telophase nucleus received a newly broken end. The broken ends fused again following the next round of replication, and the breakage-fusion-bridge (BFB) cycle continued throughout development of the endosperm. She called this sequence of events the *chromatid breakage-fusion-bridge cycle*. It took place in the male gametophyte and in the endosperm, but did not persist in the embryo. This knowledge on the behaviour of the broken ends of chromosomes was obtained by direct cytological observations, and by the use of certain marker genes affecting pigmentation of the aleurone and plant tissues (stems and leaves), and the quality of starch in the endosperm. The analysis of markers in one of the early experiments is illustrated in Fig. 4.

The chromosome with the "sticky" end carries a dominant allele (C) which gives pigmented (purple) kernels, and an allele (*Wx*) for normal amylose starch which stains blue with iodine (when the kernel is scratched and stained). The two chromosomes contributed by the female (only one is shown) carry the recessive alleles for colourless kernels (*c*) and amylopectin starch (*wx*), which stains red. The BFB cycle in the endosperm of plants of this genetic constitution results in kernels with a characteristic pattern of variegation. Off-centre breaks of the chromatin bridge between the *C-Wx* genes, in early development, result in cells in which the dominant *C* allele is missing. Subsequent cell divisions then give sectors of cells which are colourless, with the *Wx* phenotype (*cWx*). Within these sectors further breaks in the Wx-centromere region result in loss of the *Wx* allele and the formation of subsectors with the *c wx* phenotype (Fig. 4).

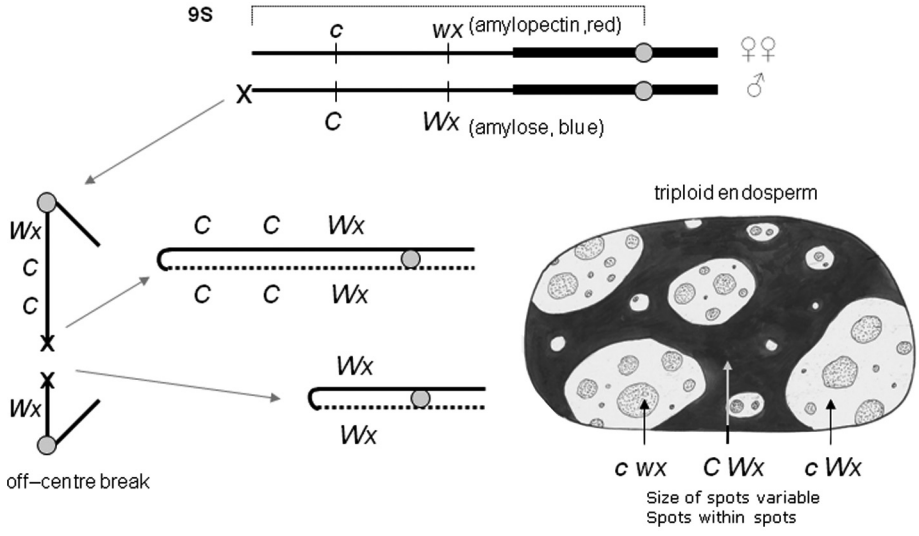

Fig. 4. Variegation in the endosperm cells due to the chromatid breakage-fusion-bridge (BFB) cycle. The kernels are purple with colourless sectors, and within these sectors are red spots (x = sticky ends)

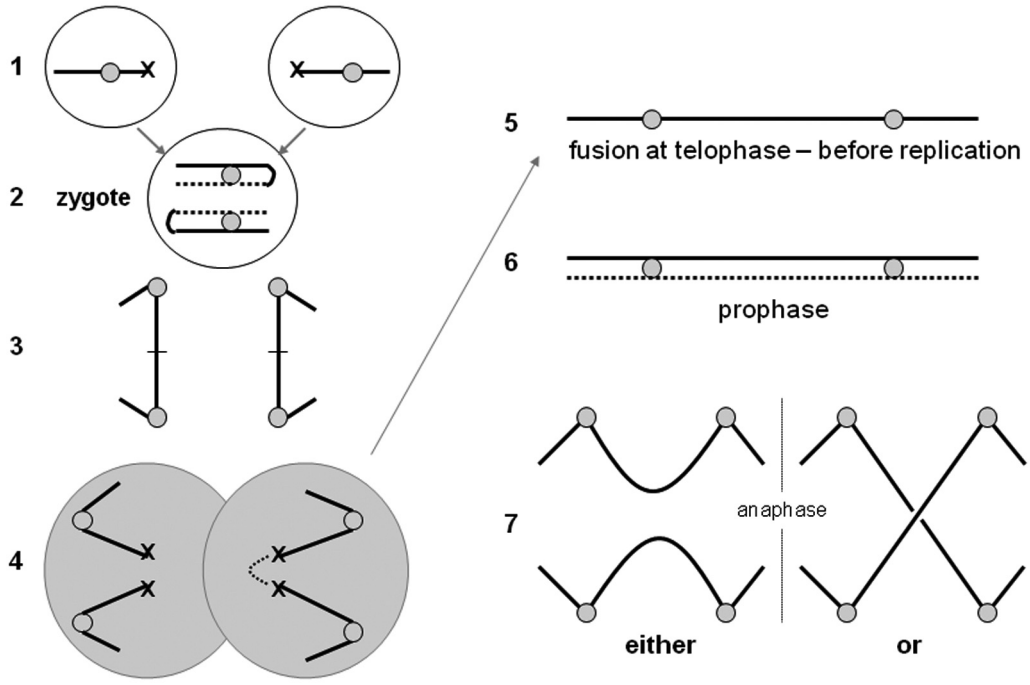

Fig. 5. The chromosome breakage-fusion-bridge (BFB) cycle in the zygote (x = sticky ends)

The characteristic feature of these kernels therefore is that they are variegated with sectors of colourless aleurone of variable size, depending upon the stage of development at which the off-centre breaks giving loss of the *C* allele occurred. Within the colourless sectors are subsectors of *c wx* phenotype (spots within spots) resulting from the subsequent loss of the *Wx* allele, which can be visualised by iodine staining after scratching off the pericarp.

CHROMOSOME BREAKAGE-FUSION-BRIDGE CYCLE

When two parent plants, which are both generating "sticky" ends at meiosis are crossed, then the zygote nucleus can carry broken ends on both homologues. The triploid endosperm nucleus from such a cross carries three homologues with broken ends and these appear to act independently, and to each go through the chromatid BFB cycle. In the developing embryo a more complex sequence of events takes place, which is known as the *chromosome breakage-fusion-bridge cycle* (Fig. 5). At first telophase following fertilisation the "sticky" ends of the two broken chromosomes join together. This joining happens before replication and thus effectively prevents the individual chromosomes from initiating the chromatid BFB cycle. The chromosome BFB differs from the chromatid BFB in that it continues during the early development of the sporophyte, and "healing" of the broken ends only takes place much later on in development. McClintock was interested in the chromosome BFB cycle because she was trying to use it to induce small internal deficiencies in 9S, for the study of induced mutations.

THE GENETIC EARTHQUAKE

In 1944 one of the crosses, in which a broken chromosome 9 was contributed by both parents, led to some unexpected results. It triggered a "genetic earthquake" in the kernels of the ear concerned. The embryos in the kernels were undergoing the chromosome BFB cycle in their early development, and when the seeds were germinated and grown McClintock saw a burst of genetic instabilities among the progeny plants. Some of the 677 kernels failed to germinate at all (87), and others gave rise to highly abnormal plants which died as seedlings (134), or as young plants in the field (73). Three hundred and eighty- three plants grew to maturity, and some of these (150) had their inflorescences fixed for pachytene analysis at meiosis, and the rest were self-pollinated.

Pachytene analysis revealed extensive genome rearrangement: deficiencies in chromosome 9; duplications of 9S; telocentrics; isochromosomes; breaks in chromosomes other than 9; translocations; inversions and knob fusions. The plants grown from the "earthquake ear" also revealed 32 newly arising stable mutants, due to small deficiencies, and several unstable mutants affecting the plant phenotype. These instabilities manifested them-

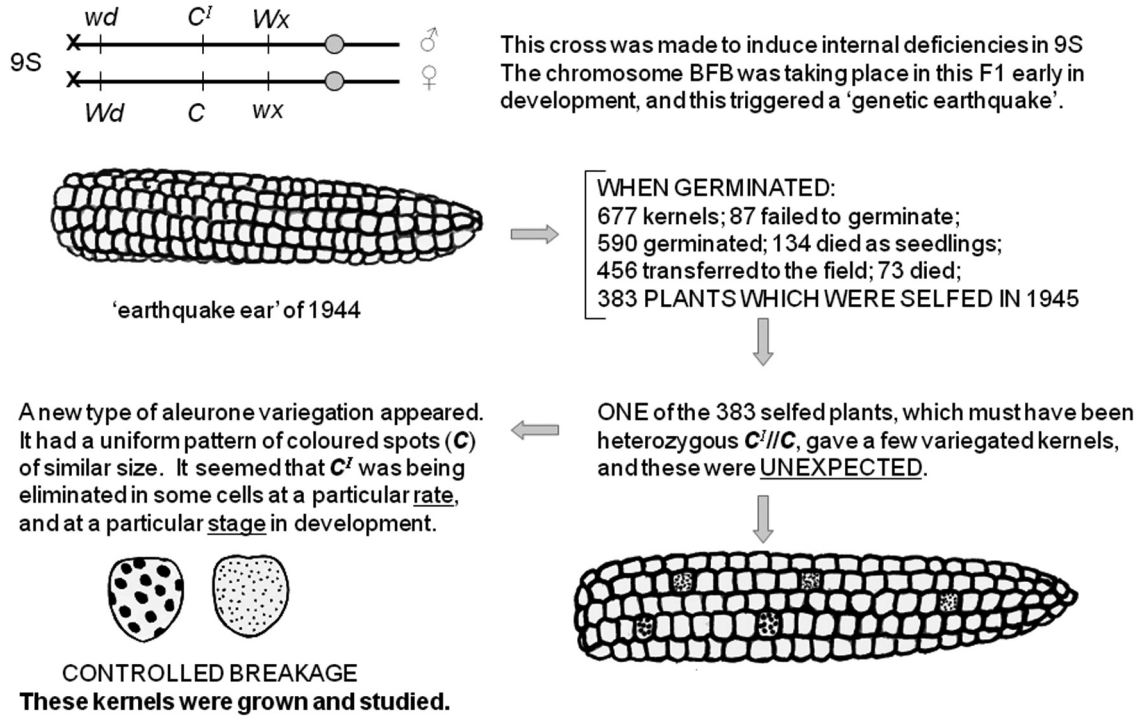

Fig. 6. The genetic earthquake resulting from the chromosome BFB cycle which led to the discovery of the *Ds* locus. Kernels colourless (aleurone) with purple spots (x = sticky ends)

selves as sectors of mutant tissue (e. g. white and yellow-green) and these mutations appeared to be controlled and to take place at specific times in development.

Of particular interest was one selfed plant which carried a few variegated kernels in its ear (Fig. 6).This plant was expected to be heterozygous C^I/C at the *C* locus, because of the way in which the original cross had been set up, and all of its kernels should therefore be colourless due to dominance of the C^I (colour inhibitor) over the *C* allele (coloured aleurone). No variegation was expected however, because in the selfing of this plant no "sticky" ends had been introduced. Nonetheless a few kernels showed a novel kind of colour variegation: they had coloured (purple) spots which varied in size between different kernels, but which were all of uniform size within a kernel. McClintock sensed that these kernels were something special. She knew, 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. From the frequency and size of the spots (Fig. 6) it seemed that the C^I allele was being eliminated in some cells at a particular rate and at a particular stage in development.

DISCOVERY OF THE DISSOCIATION LOCUS (Ds)

McClintock set out to investigate the novel and unexpected patterns of variegation due to instability at the C^I locus. She grew the kernels concerned and used the plants in various crosses in order to obtain genotypes with additional marker genes on 9S. The idea was to study the nature of the breakage events in more detail; and by having more markers she would get more precise information about the sites in 9S where the breakage was taking place. The marker genes concerned the following phenotypes:

C^I — colour inhibitor
C — coloured aleurone
c — colourless aleurone
($C^I > C > c$, order of dominance)
Sh — normal endosperm
sh — shrunken endosperm
Bz — purple aleurone
bz — bronze aleurone
Wx — amylose starch, stains blue
wx — amylopectin starch, red

In these crosses there were no "sticky" ends present in the chromosomes. When the ears matured they developed kernels with a particularly striking appearance. Variegation was present (in the plant as well) but there was only one type of sector, as if breakage was taking place in the chromosome contributed by the male parent (from the unstable line) and removing all of the dominant alleles at the same time. There were no sectors within sectors, as had been seen by the progressive removal of markers in the induced chromatid BFB cycle, and there were no "sticky" ends present to initiate the BFB cycle in the first place.

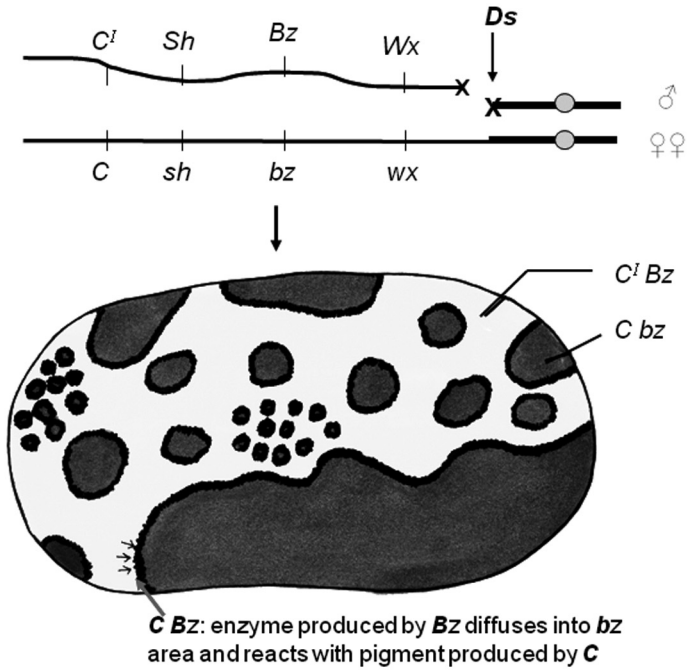

Fig. 7. Variegation in kernels produced by the action of the *Ds* locus in causing localised breakage in the short arm of chromosome 9. The kernels are characterised by a colourless background (represented as pale yellow) on which there are sectors of bronze pigmentation due to expression of the recessive *bz* allele. At the boundary of the sectors there are borders of dark pigment. This effect is due to an enzyme produced by the *Bz* allele diffusing into the *bz* area and reacting with the pigment produced by the *C* allele

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In addition it looked as if the breakage was controlled in the sense that the time of breakage (size of sectors), and the frequency (density of sectors) seemed to vary during development, i.e. sectoring was not uniform over the whole of the kernels. In some F_1 ears the majority of kernels showed a speckled appearance, due to numerous small sectors (sometimes of only one or two cells). In others there were kernels with large areas of recessive tissue and only small background patches of the dominant phenotype. In addition some kernels showed small and large areas on the same kernel (Fig. 7.). The system seemed highly dynamic and tightly controlled, and McClintock proceeded to try and work out the genetic basis of this new phenomenon.

When she looked at the pachytene chromosomes she saw breakage in the short arm of chromosome 9 in some of the plants. This breakage involved only one of the two homologues, and it appeared to always take place at the same site at the junction of the heterochromatic and euchromatic regions near to the waxy locus. It seemed as if there was a particular site of breakage, which she called the *dissociation*, or *Ds*, locus and mapped its position cytologically. Breakage was indeed removing the dominant alleles, *en bloc*, and giving rise to acentric fragments which could be seen as micronuclei in the cytoplasm. Following breakage, a chromatid BFB cycle was initiated by fusion of the broken ends of the two chromatids, but as there were no other markers in the region between the *Ds* locus and the centromere there were no detectable consequences to the BFB cycle.

What exactly was this *Ds* locus, and how was it that these breakages were controlled in relation to development? As far as could be seen most of the breakages took place late on in development; they could be found in the older tissues of the green plant, in the sporogenous cells and in the endosperm where they were most easily observed.

DISCOVERY OF THE ACTIVATOR LOCUS (Ac)

The first clue to the controlling influences acting upon *Ds* came when some kernels were found without any variegation in plants which were expected to show *Ds* breakage. Pollen of a male carrying C^IC^I *DsDs* had been placed onto the silks of a plant which was *CC dsds*, and all of the progeny were expected to be heterozygous with variegat-

ed kernels due to loss of the *C'* allele. In the event only half of the kernels turned out to be variegated and the other half were normal, a ratio of 1:1. McClintock decided, correctly as it later turned out, that one of the two parents (the male) must have been heterozygous for some other factor which segregated as a Mendelian unit and which was necessary for breakage to occur at the *Ds* locus. She called this factor the *Activator* or *Ac* element. *Ac* was inherited independently of *Ds* and acted as a dominant allele in crosses.

Dosage effect of Ac

Further evidence for the controlling influence of *Ac* came from breeding tests in which its dosage was varied in the endosperm. The endosperm is triploid, and it is therefore possible to arrange crosses such that 0, 1, 2 or 3 doses of *Ac* are present. This is done by contributing no *Ac* elements from either parent (0), one element from the male side (1), two elements from the female side (2) or one from the male and two from the female (3).

The dosage of *Ac* controlled the stage of development at which *Ds* breaks occurred: the greater the dose the longer the delay in breakage, and thus the smaller the sectors. She concluded that not only did *Ac* control whether or not breakage would occur at *Ds*, but also when it would take place. However she still could not say how it was that the frequency of breakage appeared to alter during development, and this effect turned out to be a property of *Ds*.

Change of state of Ac

The effect of *Ac* seemed to vary, so that in some kernels there were sectors which suggested that the breakage at *Ds* was taking place at different times in development in different parts of the same kernel. Furthermore the effect of *Ac* varied between different kernels on the same ear, and between the ears derived from different crosses, despite the fact that the dosage was kept constant.

Breeding tests were set up to investigate this aspect of the behaviour of the *Ac* element. A female line allelic for *Ac* (i. e. *Ac*/*Ac*, identical alleles at a single locus) was established by selfing, so that all the *Ac* elements in the F_1 heterozygote were identical (Fig. 8). Results of the cross showed the appearance of altered forms of *Ac*, as witnessed by altered times of breakage of *Ds* in some of the kernels. The effect mimicked that of different doses of *Ac* already described. Some kernels were fully coloured, showing no *Ds* breaks, and others had a few large spots of colourless tissue due to early *Ds* breakage. The majority showed patterns typical of that expected for a single dose of *Ac*. Further breeding tests indicated that the altered kernels were due to a change of state of *Ac*, and that some were also due to a change in the number of *Ac* elements. Changes of state also happened in *Ds*, but this aspect is not dealt with.

TRANSPOSITION OF Ds

While experiments were being undertaken to accurately map the position of *Ds* at its standard location an unexpected event took place at one of the loci being used as marker in 9S. The event involved an alteration in the behaviour of the *C* locus, which changed into a new colourless mutable form denoted as $c^{m\text{-}1}$ (mutable allele $c^{m\text{-}1}$), and this event marked the discovery of transposition.

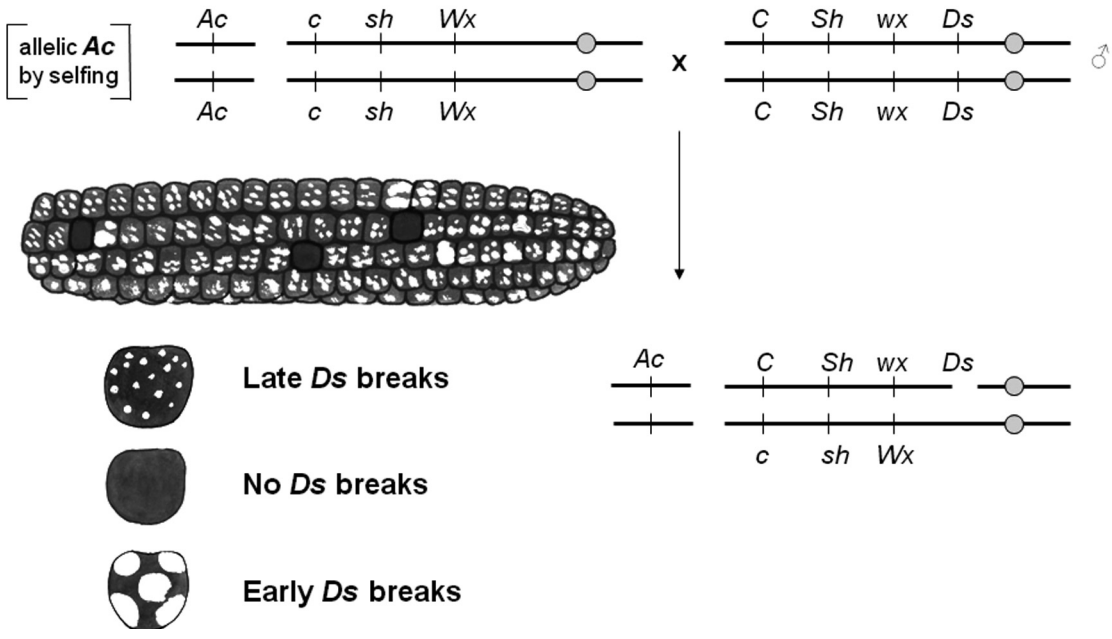

Fig. 8. Breeding tests using allelic forms of Ac set up to test for changes of state of the Ac element. Kernels are purple with colourless spots

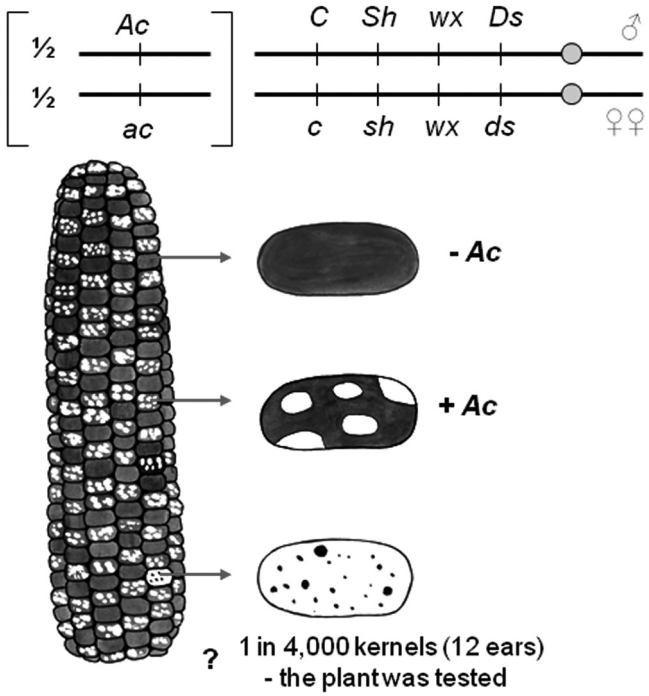

Fig. 9. An unusual kernel turning up in a crossing experiment involving 4,000 kernels revealed the phenomenon of transposition. Kernels are purple with colourless spots

A cross had been made of the type shown in Fig. 9, in which a single male was used to pollinate 12 genetically similar females. The male carried dominant markers on 9S, had *Ds* at its standard location, and was also heterozygous *Ac/ac*. The females carried the recessive alleles and had no *Ac* or *Ds* elements. From such a cross about half the kernels should show the *C Sh wx* phenotype, purple without variegation, and the other half should carry *Ac* and be variegated with colourless spots due to breakages at *Ds*. The expected classes of kernels appeared in the 12 ears with the exception of ONE kernel out of the 4,000 examined. In this kernel the variegation pattern was reversed, and it showed a few coloured spots (*C*) on a colourless background. This kernel was grown and the plant obtained from it tested in various ways to determine the cause of this unexpected "reverse variegation". The tests indicated that reverse mutations were occurring from the recessive (c^{m-1}) to the dominant *C* allele, and that the mutable condition had arisen in one of the chromosomes 9 contributed by the *Ac* carrying male parent.

Ds type activity was also present in the chromosome carrying the new mutable c^{m-1} locus. The location of this activity had also moved: it was no longer to the right of wx, as would be expected, since this was its location in both chromosomes 9 of the male parent, but its new location was inseparable from that of c^{m-1}. The site of chromosome breakage had now moved (transposed) from the standard location of *Ds* to this new location.

It was soon discovered as well that the reverse mutations of c^{m-1} to *C* (purple spots) would appear only if *Ac* was also present in the nucleus, and that the time of occurrence of the mutations was controlled by the dosage and the state of *Ac* in precisely the same way that the dose and state of *Ac* controlled *Ds* breakage in its standard location. In the absence of *Ac* neither mutations of c^{m-1} to *C* nor *Ds* breakage occurred. All of the evidence was consistent with the assumption that the c^{m-1} allele arose by transposition of *Ds* to the site of the *C* locus, and that the new mutation at *C* was caused by the insertion of *Ds* into it.

Aside from the breakage, *Ds* now had a second effect of causing mutations in *C*. At first McClintock tried to explain this mutability of the *C* locus in terms of *Ds* breakage as well, but she could find no satisfactory explanations on this basis.

The answer came from further breeding tests on some of the plants carrying the mutable c^{m-1} locus. In a few kernels reversion of c^{m-1} to *C* took place early enough in development to have affected the gametes, so that the revertant allele was passed on to the progeny, and the *Ds* element was then no longer found at the *C* locus. The locus now functioned normally, and in the presence of *Ac* the chromosomes no longer broke at the *C* locus either. *Ds* had gone!

It was concluded that the mutability of *C* was not due to the locus itself, but to the effect of *Ds* being inserted into it. With *Ds* present in the locus the expression of the dominant *C* allele is suppressed, and when *Ds* moves out expression is restored. The coloured spots in the "reverse variegated" kernels represent cell clones where *Ds* has transposed out of c^{m-1} at a certain stage of development. If the transposition occurs late on in development, after the embryo is formed, then the revertant allele is not inherited; but if it takes place in the sporogenous cells which give rise to the germ cells then some gametes will carry *Ds* at a new position (which may or may not be detectable) and their 'reconstructed' c^{m-1} (i.e. *C*) locus will be stable. The events concerning *Ds* transposition are summarised in Fig. 10. So the activity of *Ds* did not nec-

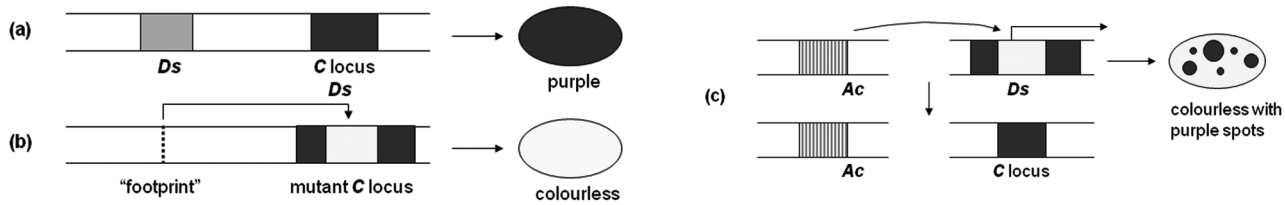

Fig. 10. Summary of events concerning transposition of *Ds* at the *C* locus. (a) *Ds* and the normal *C* locus (purple aleurone) are shown in the same chromosome. Under the control of *Ac* (b) Ds transposes and inserts into the *C* locus: the function of *C* is obliterated giving colourless kernels. (c) Further transposition events during development of the endosperm release *Ds* and give revertant cell clones in which the normal function of *C* is restored

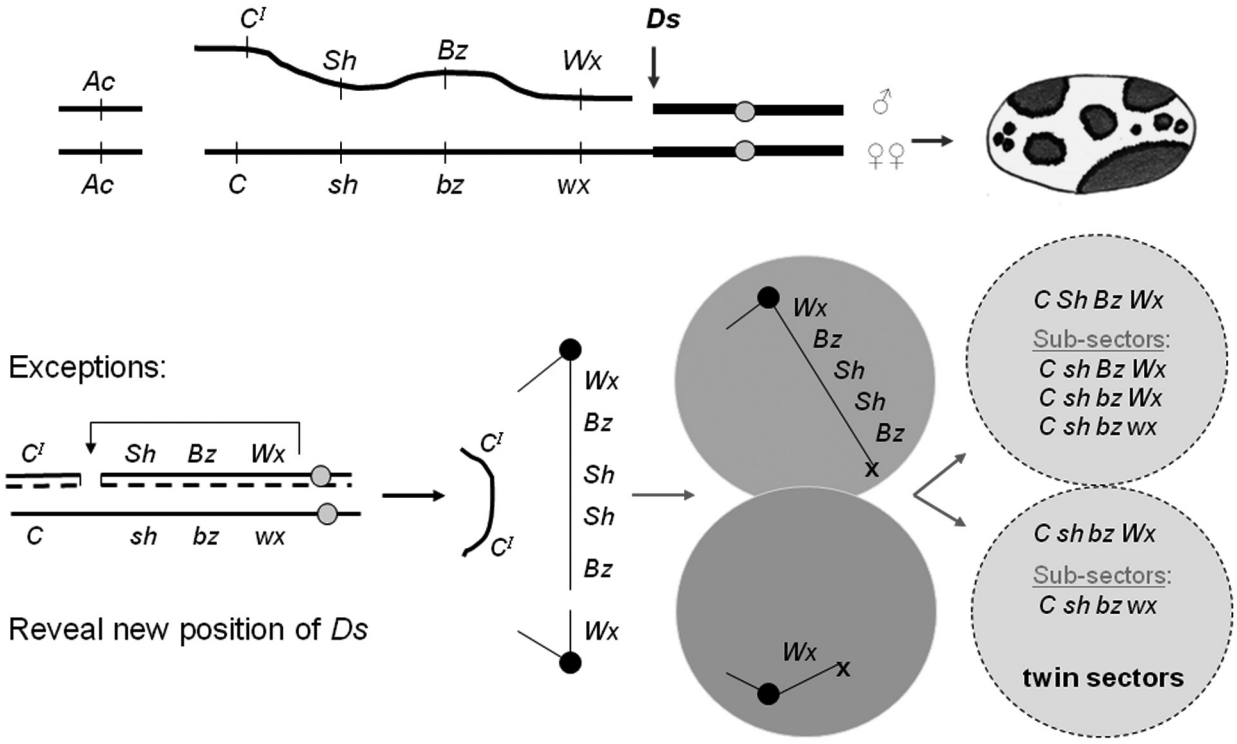

Fig. 11. Distinctive patterns of variegation can reveal new locations of Ds activity following transposition. Kernel is yellow with bronze spots (x = sticky ends)

essarily cause chromosome breakage. This was not known from studies of *Ds* in its standard location because in this position it was not inserted into a marker gene. As explained later the "state" of *Ds* could also vary, and it could either exist in a form which gave a high level of breakage or one which did not. McClintock subsequently discovered other mutable loci due to *Ds* insertions; namely c^{m-2}, wx^{m-1}, bz^{m-1} and bz^{m-2}. She also noted that when *Ds* transposed out of a locus the expression of that locus could sometimes be permanently changed, as if the transposed element left behind a "footprint" in the form of a new allele.

Mapping Ds

Once it was realised that *Ds* was a mobile element its movement to new locations could be detected in several ways. (i) The origin of new mutable loci, such as c^{m-1}, revealed its insertion and marked the locus concerned; (ii) a new position was often discovered by the occurrence of kernels with new patterns of variegation, as in Fig.11.

In this instance, where *Ds* has been transposed to a position between C^1 and *Sh* there is immediate loss of the dominant allele following the breakage event. The chromatid BFB cycle then takes place and off-centre breaks give distinctive patterns of sectoring, with a series of subsectors within sectors due to sequential removal of markers as the cycle proceeds. (iii) The position of *Ds* could be mapped by standard methods of recombination analysis, because in the absence of *Ac* it is completely stable. Fig. 12 shows a map of many different sites to which *Ds* has transposed within the short arm of chromosome 9. With an adequate means of detection it is possible to show that *Ds* can transpose to numerous other sites as well within the chromosome complement.

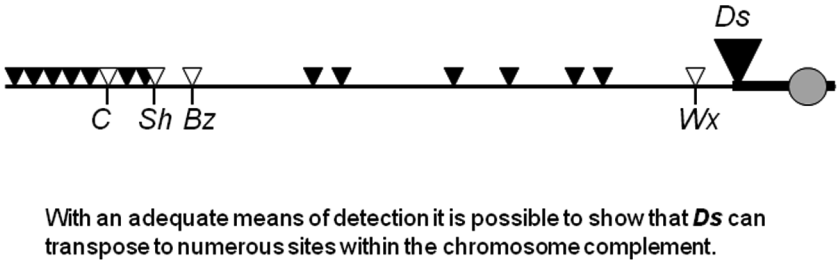

Fig. 12. A map showing the approximate positions of several sites of *Ds* activity that have been mapped in the short arm of chromosome 9

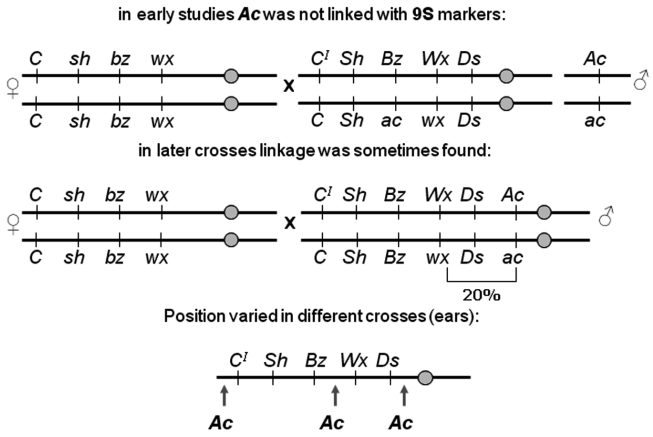

Fig. 13. In some crosses *Ac* showed linkage with marker genes in chromosome 9, and it could occupy different positions in the short arm

TRANSPOSITION OF Ac

It subsequently transpired that *Ac* could transpose as well. When *Ac* was first discovered its position was unknown, except to say that it was not linked to any of the markers on 9S; and because the other chromosomes were less well marked it could not be located in the early work. Eventually, in one of the crosses, it appeared in chromosome 9, and was found to be about 20 crossover units to the right of wx (Fig. 13).

It was later mapped to various other locations in 9S, and when transpositions did occur they usually happened in only 2–3 % of the sporogenous cells so it was stable enough to be mapped by recombination analysis. Alterations in the dosage of *Ac*, which turned up unexpectedly, could now also be explained in terms of its transposition. The mobility of the element means that it is not restricted to occurring at allelic sites only, as is the case with a normal gene.

AUTONOMOUS AND NON-AUTONOMOUS ELEMENTS

The principal features of the *Ac-Ds* family of McClintock's controlling elements are summarised in Fig. 14. *Ac* is an autonomous element that controls its own transposition. It can also cause gene mutations and chromosome breakage. *Ds* is a non-autonomous element under the control of *Ac*.

McClintock looked upon these elements as small submicroscopic segments of chromosomes with "cohesive ends". She thought that they played an important role in the control of development, and that the chromosome breakages which they induce have a long-term evolutionary significance leading to restructuring of the genome.

It is not entirely clear what causes the elements to undergo changes of state. McClintock viewed these effects as a property of the elements themselves which is associated with the regulation of development, and she went as far as to demonstrate particular stages in development where such changes did occur.

Other workers are of the opinion that changes of state represents differences in gene expression due to insertion of the elements at different sites with a gene, but this view seems to overlook the developmental component. Another theory invokes changes in methylation patterns at target sites within the elements, or even a dual role of the transposase protein which may double-up as a repressor of transposition.

CLONING AND MOLECULAR CHARACTERISATION

In the 1970s the *Ac* and *Ds* elements were cloned. This was done by reverse transcription and by using cDNA clones as probes to fish-out the genomic sequences from a library of cloned DNA fragments (Fedoroff, 1984). *Ac* is a complex genetic element of 4,563 nucleotide base pairs (nbp), and it ends with 11 bp inverted terminal repeats. A target site of 8 nbp at both ends of the element is duplicated during its insertion. Most of the length of the element is accounted for by a single transposase gene with five exons. There are several *Ds* elements which vary in length, but which are closely related to *Ac* and have the same 11 bp inverted terminal repeats. *Ds* elements are shorter than *Ac* and are derived from it by internal deletions which inactivate their transposase gene. The trans-acting control of *Ac* over *Ds* is therefore due to the fact that *Ac* has the active gene and *Ds* does not. "Footprints" left behind by removal of the elements have also been cloned. An element apparently leaves a locus in a slightly different form to that which was present when it inserted itself.

ORIGIN OF CONTROLLING ELEMENTS

Where did the *Ds* and *Ac* elements come from? McClintock thought that the chromosome BFB cycle that she initiated in 1944 generated the elements through rearrange-

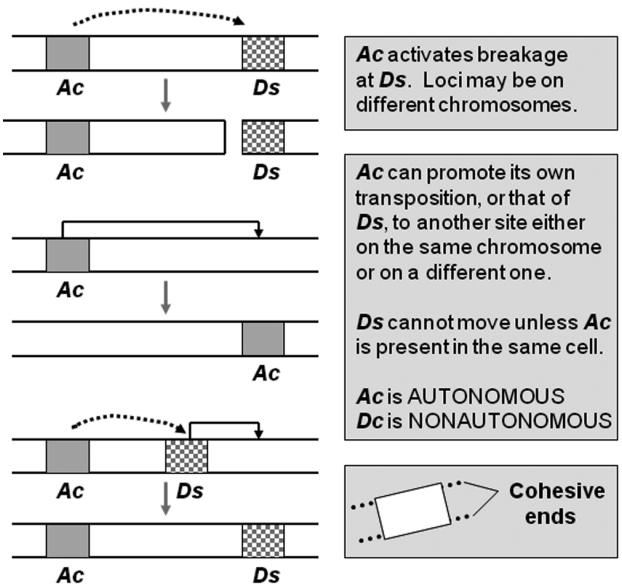

Fig. 14. Summary of the principal features of the *Ac-Ds* family of controlling elements

ments of the genome which released them from a quiescent state where they had been lying buried in heterochromatin, and this is probably what happened.

CONCLUDING REMARKS

The *Ac-Ds* family is but one of several families of transposable elements in maize. Others are *Spm-dSpm*, worked on by McClintock herself, and *Dt* which was identified and studied by Marcus Rhoades (Rhoades, 1945). A detailed list of all the known maize DNA transposons is given in Fedoroff 1983. McClintock's Elements, like those in other species and other systems in maize, are scattered throughout the genome: some even have cis-acting alterations to their vital parts which render them immobile and "dead". Those that can mobilise do not move about randomly, but are normally static and quiescent. Mobilisation is triggered by some drastic stimulus to the genome (the chromosome BFB cycle!), such as wide hybridisation or extreme environmental stress (callus culture). What then happens is a burst of genetic instability in the form of gene mutations and chromosome breakages, which can lead to gross structural rearrangements in the genome. Transposons can thus have a long-term evolutionary role by providing a mechanism for rapid change in response to sudden alterations in environmental conditions. McClintock set great store by this capacity of controlling elements to generate variation and to facilitate genome evolution (McClintock, 1978). She also firmly believed that her controlling elements had a major role to play in regulating the process of development, because of the way in which they act at particular times, and the way in which their action appears to follow a programmed pattern, but this aspect of her work has not been accepted. This account tells only the beginning of the story, that of the discovery of DNA transposons, which is the part that is usually least well narrated

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ОТКРЫТИЕ ТРАНСПОЗОНОВ КУКУРУЗЫ БАРБАРОЙ МАК-КЛИНТОК

Джонс Н.

❀ **SUMMARY: В статье описан ранний период работы Барбары Мак-Клинток на ДНК-транспозонах кукурузы, когда она открыла Ac-Ds семейство мобильных «контролирующих элементов». Вначале приведены цитологические особенности системы, которая была использована для создания интактных хромосом с «липкими» (обломанными) концами. Затем описаны цитогенетические аспекты циклов разрыва-слияния-мостика на хроматидном и хромосомном уровнях, показывающие, как мозаицизм семян кукурузы может быть объяснен разрывами хромосом. Введение разорванных хромосом от обоих родителей в зиготу инициировало «генетическое землетрясение» 1944 года, которое привело к открытию Ds и Ac. Обнаружение способности Ds менять свое положение на хромосоме произошло благодаря счастливой случайности: транспозиция была обнаружена в экспериментах, направленных на точное картирование Ds. Похожий случай продемонстрировал и транспозицию Ac, и затем взаимоотношения между этими элементами были исследованы с точки зрения их автономной и неавтономной природы.**

❀ **КЛЮЧЕВЫЕ СЛОВА:** ДНК-транспозоны; Мак-Клинток; цикл разрыв-слияние-мостик; элементы Ac-Ds; цитогенетика кукурузы.

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