ExcellGene

FROM CORN TO CHO TRANSPOSON-TECHNOLOGY

Maturing a concept over decades

Part 1 of 4

1974: Institute for Genetics, Justus-Liebig University, Giessen, Germany.

Prof. Fritz Anders (1) passes two papers to his student: "*Please read these (2, 3)…. and provide a summary of them next week in our Journal Club!*" Published two decades earlier and authored by Dr. Barbara McClintock (1902-1992), the papers were not an easy read* and accepting their implications was even more challenging, especially for a 25-year-old. McClintock, then already a member of the National Academy of Sciences in the US, had to be taken seriously. She demonstrated that **genes on chromosomes can change their place during the early development of a young plant – the growth of a kernel on a corn cob!** This phenomenon, highlighted by a change in pigmentation of the kernel, is referred to as **transpositions**. Two concluding sentences of the papers are here:

*"There can be little question that **transpositions** of both Ds and Ac occur. These units may be **transposed from one location to another within the chromosome complement**."*

* It has been concluded that the changed phenotypic expressions of such loci are related to changes in a chromatin element other than that composing the genes themselves,and that mutable loci arise when such chromatin is inserted adjacent to the genes that are showing the variegated expression.

The geneticists in the room were surprised. "*Genes can go from one position in a chromosome to another one, they can jump around?*" Trained in classical genetics and chromosome structures as established by Gregor Mendel (4) and by Thomas Hunt Morgan (5), the thought of genes being mobile on chromosomes was very strange. **Fig. 1.1** shows a diagram of Dr. McClintock with a corn ear in her hands exhibiting about 150-200 kernels with dark and light pigmentation. In **Fig. 1.2** a kernel diagram with an aleurone layer of cells is presented and **Fig. 1.3** reproduces a microscopic image of a thin cut through the surface layer of the kernel, frequently transparent, and the aleurone layer, with pigmented cells.

Dark pigmentation in the aleurone layer is dominant (most frequent) in wild-grown corn – and white/yellow ears are mainly from breeds for consumption. The two papers explained something in-between: "Variegated" coloration patterns on individual kernels as shown in **Fig. 2**. The pigment in aleurone cells is anthocyanin and a gene involved in controlling its synthesis is located on Chromosome 9 of the plant. Maize has 10 pairs of chromosomes, thus 20 chromosomes after fertilization*.

Each kernel is a young plant. Thus, on a single corn ear McClintock could analyze the result of hundreds of fertilizations – and of course on a maize field she could eventually collect data from tens of thousands of kernels.

*To simplify, the authors decided not to mention triploidy of parts of the seed tissues.

Fig. 2
Part of a corn ear showing many kernels with variegated colorization in their aleurone layer. Image from https://www.cshl.edu/the-secret-history-of-corn-is-revealed-in-its-genome/

FIGURE 1

Fig. 1.1
Drawing of Dr. McClintock holding a corn cob, showing different coloring of kernels, resulting from crosses between different parental plants.

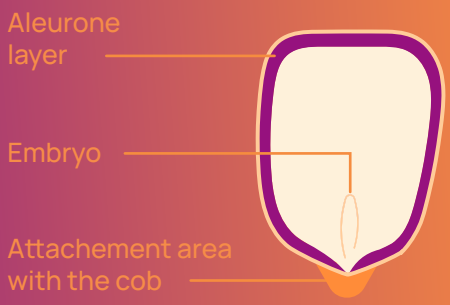


Fig. 1.2
Diagram as a vertical cut of a kernel. The blue pigmentation is made by cells in the aleurone layer.

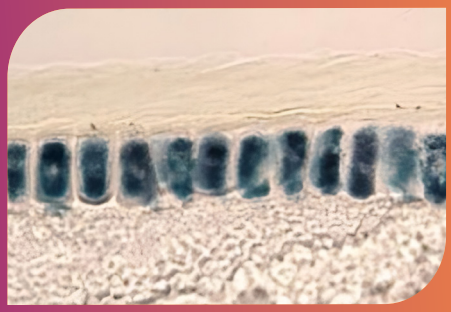


Fig. 1.3
Microscope image of a thin cut of a kernel. The pigmented cells are aleurone layer cells (image from Chatham et al. 2019, https://doi.org/10.1007/s00122-019-03414-0).

Genetic foundation of variegated pigmentation

The 1950 and 1953 publications demonstrated that a variegated appearance of a kernel is caused by a genetic element “Ds” that has transposed out of a C’ gene on chromosome 9. McClintock knew that C’ is a “strong” (=dominant) regulatory gene allowing the production of anthocyanin in cells. What is “dominant”? Since cells after fertilization have always two copies of each chromosome, whether only one or both chromosomes have C’ does not matter - the cell will produce the pigment anthocyanin. Ds transposed into C’ makes it non-functional, i.e., the cells will not produce pigment. A weak gene (genetic term “recessive”) version of C’ is C. When both chromosomes contain the recessive C then the cell will not produce pigment **(Fig. 3.1)**. When however, one chromosome carries C’ then the cell will produce anthocyanin **(Fig. 3.2)**. In **Fig. 3.3** the effect of Ds transposing into C’ and out of C’ is shown, with the “on” and “off” status dependent on where Ds is located. **(Fig. 3.4)**

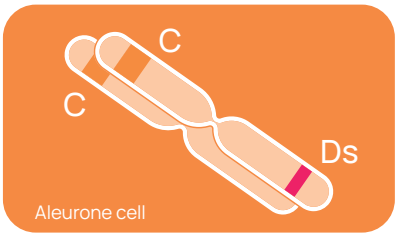


Fig. 3.1
Aleurone cells showing a pair of chromosomes 9 with the two recessive genes on the pair of homologous chromosomes and the Ds gene (red) on one of the chromosomes but distant from the C locus.

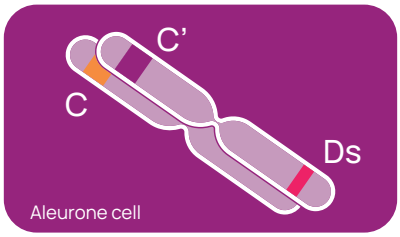
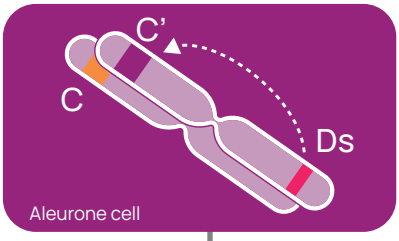
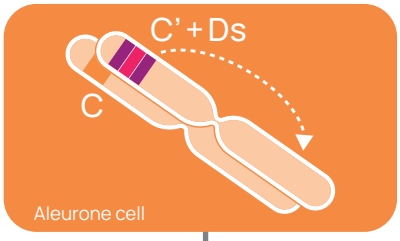
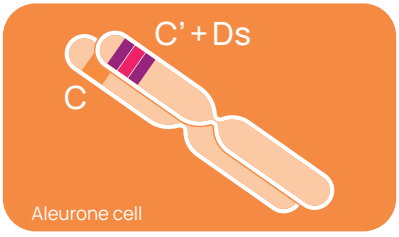


Fig. 3.2
Aleurone cells showing C and C’, with Ds outside of the C locus

FIGURE 3







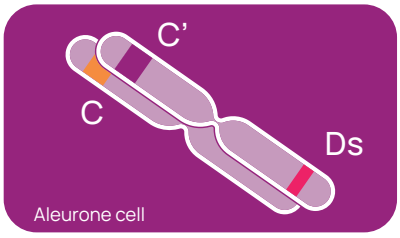
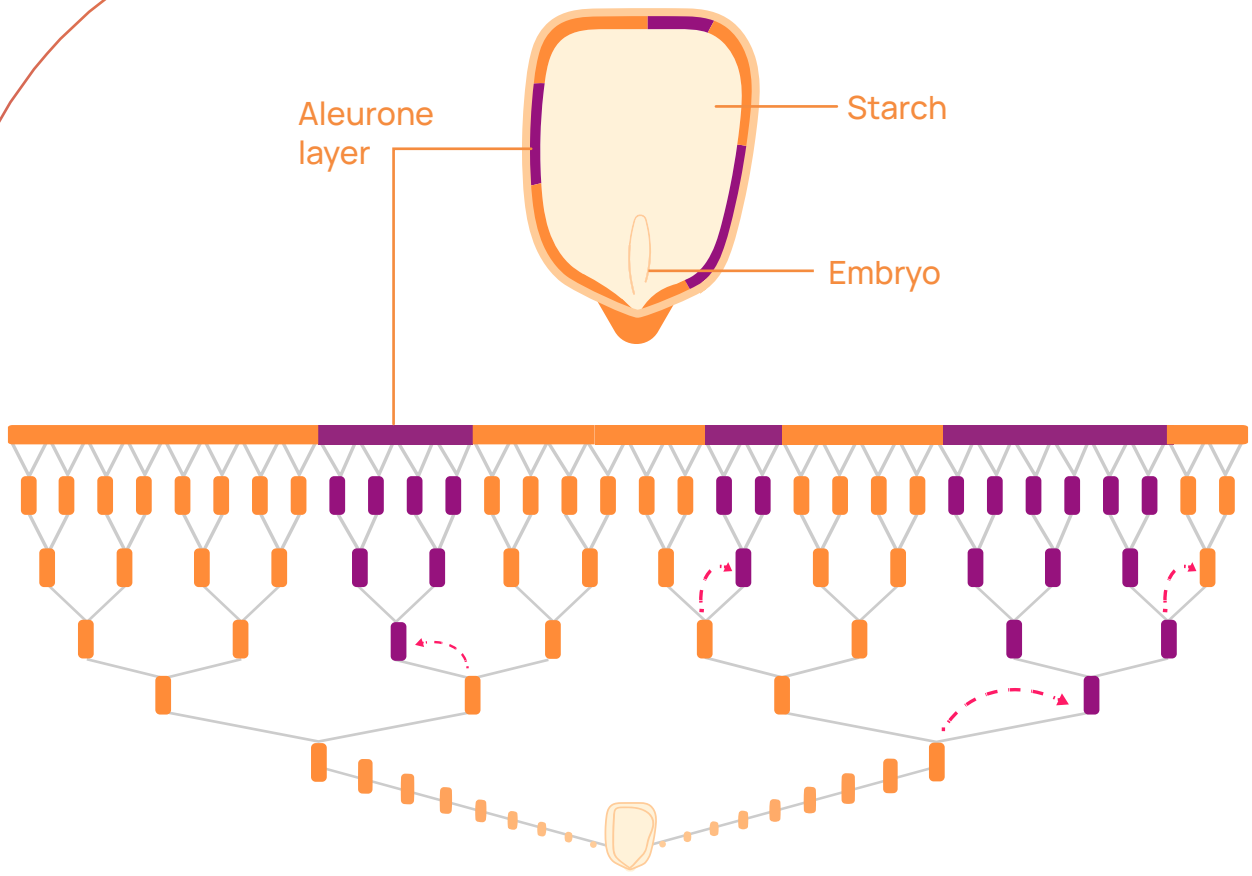


Fig. 3.3
Effect of transposition of Ds. Ds inserts into C’ and inactivates it, the cell turns yellow.

Fig. 3.4
Effect of transposition of Ds. C’ has been inactivated by insertion by insertion of Ds. When Ds transposes out of C’, then pigmentation of the cell occurs.

FIGURE 4



The diagram in **Fig. 4** tries to capture events over time during the development of a kernel while aleurone cells are dividing. Cell divisions and the timing of transpositions are shown which eventually result in the partially pigmented aleurone layer **(Fig. 1.3)** of a ripe kernel.

McClintock also found that the frequency of transpositions is influenced by the presence of yet another DNA element (Ac), also capable of transposition. Ac is often located on the same chromosome as Ds. We know now that Ac encodes a transposase – an enzyme that can cut itself out of a DNA molecule and transpose its' DNA into another site. The same transposase can also initiate the transposition of Ds. Ds has a similar DNA sequence to Ac, but the transposase is not functional (deletion of a part of its' DNA) (6). Therefore, transposition of Ds can be initiated by Ac. Multiple copies of Ac copies can be in the genome and more Ac elements will induced more frequent transpositions. We understand now: More Ac genes produce more transposase enzyme, and thus more events are triggered. Transpositions occurring at different times in the development of the kernel will result in many spots of different sizes.

Fig. 4
A simplified scheme of events in a kernel and its aleurone layer: Cells of the future aleurone layer divide. The cells are yellow initially because the C' is inactive due to insertion of Ds. The other chromosome carries the weak (recessive) C gene. During kernel growth in some cells Ds jumps out of the C' gene. Early events will result in large, pigmented areas, late events in smaller ones, as seen in the figure 2. Here 4 transposition events are indicated (red stippled arrows), but many more can occur. Only 4 cell divisions are indicated – more must be considered. In the scheme, Ds also jumps back into the C' gene and thus turns the subsequent cells pigment-free.

Patterns of colored and non-colored sections within a kernel, their size and their number generated during the 2-3 months of maturing gave important information on the frequency of transposition.

How did McClintock conclude on transpositions of Ac and Ds? This is too complicated to explain fully in this short text. However, her interest and work over many years on chromosomes, a highly relevant topic 1940-50s in biology, was crucial. She had analyzed structures and relationships between chromosomes and kernel appearance. She executed many genetic crosses between corn types with unique pigmentations of kernels. By microscopy of cells in the aleurone layer she could see chromosome modifications based on some of these crosses ("fragile site"). Some chromosome breaks correlated in timing with the unique variegated coloration patterns: When seeing chromosome breaks in very young kernels, large-pigmented areas would develop. This observation led her to further her research culminating in the two papers quoted. Subsequent research revealed transpositions occurring frequently in and out of many genetic sites in chromosomes of corn and in other parts of the plant (7).

Mobile genetic elements are everywhere

Today, we use the term "jumping genes" or **transposons** for this type of DNA, and we know that bacteria, plants, **and** animals - all have such DNA elements. The monumental insights by Dr. McClintock, who had dedicated her life to the genetics of corn, were shared with the scientific public before we learned about the structure of DNA from Watson and Crick (8), the cloning of DNA in plasmids by Cohen and Boyer (9), and many other revolutionary insights in molecular biology. It would earn McClintock the Nobel Prize in 1983, at the age of 81 – as a single recipient (10)! Thus it took decades for these insights to become main-stream understanding in genetics and DNA mobility.

McClintock did not complain about having to wait so long. In an interview with the New York Times newspaper, she said: "The prize is such an extraordinary honor. It might seem unfair, however, to reward a person for having so much pleasure, over the years, asking the maize plant to solve specific problems and then watching its response." Acceptance for her finding was only solidifying after transposons had been found in the animal kingdom – essentially everywhere when looked for it carefully. Further explanatory reading on McClintock's work is found in publications by Nina Federof (11) who continued to study transposases in corn. The precise sequences of Ac and Ds have been published now. Ac is about 4500 basepaires long and encodes a functional transposase. Ds is derived from Ac by mutation showing a non-functional stretch of DNA (6).

The impact of mobile genetic elements in evolution – and many different types have been identified now – is enormous. Retrovirus elements, retrotransposons, DNA transposons, Alu-sequences, etc. are considered drivers of diversification of species and they can accumulate in the DNA of a species to a nearly unlimited number (12). They remain, while found in all branches of life, a complicated and most controversial topic for research today. The term "junk DNA" has been used and discussed extensively (13), also popularized in the best seller "The selfish gene" (14).

Astonishingly, these elements are the dominant part of genomic DNA in higher animals and plants. The emerging genome sciences, thankfully able to provide rapidly and relatively cheap the entire DNA sequences of an organism, were revealing: The human genome (as those of all mammals) encodes only a small number of protein-encoding or otherwise functional genes (about 25'000). On the other hand, more than 90% of the DNA seems to be non-functional and/or highly repetitive. In the African lungfish – a very old predecessor of terrestrial animals – a genome size 14 times larger than that of humans was found. A recent publication shows that a small fern (typically 15 cm high) has the largest genome with a total DNA content 50 times larger than that of humans (15). The enlargement from smaller genomes of earlier predecessors is attributed almost exclusively to transposable elements (16). The discussions on the purpose and functionality during evolution of transposons are ongoing today, debating benefits and potential drawbacks of such DNA. It is clear however that the faithful functionality and inheritance of transposons – as "parasites of DNA in cells" was maintained over very long evolutionary time scales. In fact, the Ac transposon has been experimentally transferred into Tobacco cultures and as well into the plant Arabidopsis. In both species Ac transposes efficiently (17).

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This is a multi-part reminiscence on the emergence of an idea/concept. It is the result of many contributions by scientists over decades. The here provided text and images were executed with the help of Maria Wurm, Divor Kiseljak, Paco Pino, Concetta Cardone, Sergio Da Costa, Stéphanie Anchisi, Sebastian Rheindorf-Zaorski, Diogo de Jesus and Florian M. Wurm.
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