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# What do we still need to know about transposable element *Ac*?*

(Barbara McClintock; maize; transposable elements; evolution; genome rearrangements)

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SUMMARY

Transposable elements, originally discovered by Barbara McClintock, have been shown to occur in many if not all organisms. Their roles as selfish DNA (probable), as a major agent in evolution (unlikely) and as agents for the response to genomic stress (unclear) are discussed. Among the problems presently addressed are the mechanism of transposition and the regulation of transposition rate. The latter seems to differ in the *Ac* element of *Zea mays* compared to other transposable elements. The tendency of *Ac* transposase to form large aggregates is described, and the possible involvement of these aggregates in the control of the transposition rate is discussed.

It is the purpose of this symposium to look back on some of the important discoveries of genetics 40 years later. This can also be done for the history of transposable elements that even preceded the elucidation of DNA structure in 1953 (reviews: Döring and Starlinger, 1984; 1986; Nevers et al., 1986; Fedoroff, 1992; Gierl and Saedler, 1992; Saedler and Starlinger, 1992).

## The work of one remarkable geneticist: Barbara McClintock

As everybody knows, the early history of this field is the history of the work of only one remarkable scientist: Barbara McClintock. Her achievements have become part of a legend, and legends need to be scrutinized from time to time in order not to allow them to deviate from historic reality. It is certainly no deviation to state that the identification and early study of transposable elements by one single scientist working all by herself and with an organism that allowed only one generation per year, was and is most admirable. Her ability to pick a non-obvious and complicated problem that was not recognized by mainstream biology and to develop it to a large degree of sophistication was and is a great scientific achievement.

Particularly one point deserves a consideration of its own: was the loneliness of Barbara McClintock during her work only an impediment, or was it, on the contrary, an asset? Is it conceivable that such a development needs the continuous attention of a scientist who is neither constrained by too many administrative duties like writing grant applications or finishing a paper by the end of the grant period, nor by the necessity to supervise many graduate students and thus loosing touch with the original experiments done by one's own hands?

I would not like to say that we all should work in the manner of Barbara McClintock. We are all biologists and know that nothing can claim to be the only correct behaviour. If all of us worked without graduate students and without developing the kind of interaction in which science is propagated, science might miss something. However, our present way of doing science is so different that a strong injection of the working and thinking habits of a person like Barbara McClintock would be certainly quite beneficial.

The legend not only says how important McClintock's work was, but it also says that McClintock was not recog-

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nized for it. This is only partly true. After all, she had been president of the Genetics Society of America and was a member of the National Academy of Sciences at the time she started her work on transposable elements, and these honours were bestowed on her in recognition of her work on the cytogenetics in maize. When she began working on transposable elements, this did not become a field for very many scientists, and we cannot be surprised, because just at the same time molecular genetics had its amazing successes and understandably attracted the majority of young researchers interested in genes. Still, there were some geneticists, and quite well known ones, like M. Rhoades, R.A. Brink, and P. Peterson, who joined McClintock in her efforts to understand transposable elements.

More important than these reflections on persons, however, is our assessment of the impact of transposable, controlling elements on our understanding of biology in general. Regarding this importance, I have more questions than answers.

It is often said that McClintock's transposable elements have shaken a dogma, namely the conviction that genes are fixed in their position on a chromosome and have to stay there. That may be true, but what does it mean? On the one hand, transposable elements cause mutations. These can be compared with other mutations, like translocations that had been known before these elements, to a large extent due to McClintock's own work on maize, but also due to the work on *Drosophila* genetics. Thus, it had been known that the genome can be restructured. Transposable elements are smaller than chromosome arms and their effects can be more likened to single gene mutations, but that is not a principal difference. On the other hand, even today not much consequence is derived from the statement that a gene resides in a certain location (if we neglect the differences between eu- and heterochromatin which have nothing to do with transposons). Therefore, the movement of an element from one place to another, interesting and sometimes conspicuous as it is, does not explain much to us even today.

McClintock herself has pointed to the fact that the behaviour of transposable elements is 'programmed'. This sometimes gives the impression that the movement of transposable elements is inherent to the plant and may be of a yet undiscovered importance. However, we must not confuse the control of the frequency of transposition in a given tissue with the result of the transposition event. The former seems to be dictated by the elements themselves and also by their present position in the chromosome, and is only incompletely understood. The rare excision event of a transposon in a given cell and the site of reinsertion (if there is any) is a chance event. The alteration of transposition frequency after a 'change in state' of a transposon is thus not too different from the change in mutation frequency in *E. coli* after the introduction or activation of a mutator gene.

Do transposable elements have any important role in the development of the plants? Most probably not, because for each single transposable element there is a majority of plants in which this element or at least its activity cannot be demonstrated. These plants, however, develop and grow quite normally and cannot be distinguished from their sibs possessing the element.

The elements are, in special circumstances, capable to control gene activity. Specifically, if an element is inserted near a gene, the effect on gene activity may be absent or only small, but if a second such element is crossed into the progeny, the effect of the first one may become pronounced. In some of the clearer cases, this is due to the binding of a transposon-encoded protein molecule to the element near the indicator gene and an influence exerted on gene expression by the bound protein molecule. It has often been speculated why this discovery did not attract the same degree of attention as the discovery of the control of the *lac* operon in *E. coli* by Jacob and Monod. However, usually it is not stressed in these discussions that the *lac* operon is an important part of the genetic apparatus of *E. coli*, and its control by understandable environmental stimuli is part of the physiology of this bacterium. The control of genes by transposable, controlling elements in maize is a consequence of a mutation that had to occur first and had to move one of these elements into a gene, or at least into its vicinity. Thus, this gene control was not an intrinsic property of a gene, revealing something about the ways in which different parts of the organism operate or react to the environment. Rather, it was a complication of a mutational event. If I understand the history of this discovery correctly, it took a while both for Barbara McClintock and for her followers to appreciate these points clearly.

**Possible functions of transposable elements**

If transposable elements are agents for mutation rather than agents for the normal functioning of an organism, than the meaning of these elements might be found rather in evolution than in physiology. Here, if I see it correctly, we have to deal with three propositions: (*1*) The transposable elements are 'selfish DNA'. (*2*) Transposable elements are entities in population genetics and contribute substantially to evolution. (*3*) Transposable elements are agents for the rapid restructuring of the genome in response to 'genomic stress'.

The 'selfish DNA' hypothesis is an interesting default hypothesis: It potentially explains that an amplifiable and transposable DNA segment should survive chance deletion better than unique DNA, even if it does not confer any selective advantage to its host organism. If the 'selfish

DNA' hypothesis is the only way to explain the persistence of transposable elements, these elements could command limited interest at best.

Of course, even if transposable elements behave in a selfish way, this does not exclude the possibility that a selective benefit does exist for the host, too. An interesting example is the adaptive value of multi-resistance plasmids that carry transposon-borne resistance genes towards multiple antibiotics which might occur together in nature or in hospital wards. S. Cohen, H. Saedler and others have shown many examples where bacterial insertion elements serve a role in restructuring bacterial plasmids quickly enough to be observed in the laboratory.

While the findings mentioned above come from the laboratory and are based on experiments, they are subject to the limitations of space and population sizes used in the laboratory. A number of population geneticists have tried to overcome these limitations at least theoretically by formulating equations describing the potential movements of transposable elements under hypothetical conditions through larger populations and time intervals. While these considerations are quite interesting, it must always be borne in mind that a confirmation by experiment would be most helpful and that one should be aware of the possibility that the situation found in real life might be more complicated than the models based on the limited knowledge available at a certain time.

While transpositions please the evolutionist, who believes that evolution is every alteration of gene frequencies in populations, other biologists might ask for more. They are interested whether organisms differ from each other in meaningful ways, e.g., how they acquire capabilities not possessed by their progenitors, possibly enabling them to live in a new habitat. Are such alterations brought about by transposons? If so, then this has not yet been demonstrated. The well-known mutations caused by transposable elements are in their majority gene inactivations. I am not aware of cases in plants, where a silent gene becomes turned on or is allowed to be expressed in another tissue through the insertion of a transposable element, and I am also not aware that the well-studied plant genes under elaborate expression control show signs of the presence of transposable elements in their vicinity. It may be different with the transposon footprints, a peculiarity of plant transposons which insert one or two amino acids into coding regions, but again, I do not see much evidence in the sequence of plant genes that seems to indicate that these genes show a high incidence of the ensuing two amino acid duplications. Thus, even the footprints may be no more than an additional component in the everlasting process of the creation and counterselection of point mutations.

As a third point, we should consider the idea that transposable elements are important in restructuring the genome of their hosts, and this not only in a mutation-like fashion, but rather in response to certain stimuli that McClintock has called 'genomic stress'. Here, as in the former considerations of the role of transposable elements, it is not quite clear in what time scales one has to consider these effects. A response of stressed cells or plants should ensure survival in the adverse environment only, if this environment does not kill immediately and if it is present for a time long enough to make a long-term adaptation to it beneficial. Unfortunately, both of these conditions are usually not met, and most of the examples that McClintock herself has quoted do not address these long-term effects. On the contrary, the stresses quoted by McClintock are usually of short duration. Often, they can be equated to or at least correlated with chromosome breaks, as is the case both with McClintock's seminal experiments causing controlled anaphase bridges of the short arm of chromosome 9 of maize, and the several experiments in which plants were exposed to ionizing radiation. Both effects cease after a very short time. The activated transposable elements, however, that had been silent up to the time of the stress remain active thereafter for an undefined period of time, as she herself demonstrated in her experiments with *Ac* and *Ds*, as well as with *En/Spm*.

McClintock (1984) has discussed 'genomic stress' in a way that puts the activation of transposable elements and the consequences thereof in a row with more physiological reactions like the adjustment of the amount of rRNA in *Drosophila* or gall formation in response to egg-laying in several plants. In her own words, 'maize transposable, controlling elements [are able] to integrate the activity of one gene with that of another' (McClintock, 1980). She also quotes examples of speciation in response to interspecific crosses and states that these are often accompanied by genome restructuring. These ideas are expanded even more boldly in the secondary literature on this subject (Keller, 1983; Lewin, 1983).

Here, as in the case of the normal controlling function of the transposable elements, we must be cautious. It has been proven by McClintock and her successors that transposable elements can provoke chromosomal aberrations. It has not been demonstrated that these alterations are more than different kinds of mutation brought about by a potent internal mutagen. And particularly it has not been shown that the mutations caused by the transposable elements are in any respect adaptive with regard to overcoming the 'genomic stress'.

Another example of 'stress' is offered by cells which are taken from plants and put into tissue culture, often after protoplasting. Lee and Phillips (1988) have discussed whether the chromosomal aberrations seen under these circumstances are caused by late replication of heterochromatin followed by chromosome breaks due to the

rupture of anaphase bridges. They explicitly mention that under these circumstances not only all usual types of mutations occur, but also that silent transposable elements are activated, similar to the findings by McClintock leading to the discovery of transposable elements. Chromosome breaks not only can explain various types of point mutations (by error-prone repair) and chromosome aberrations. If methylation keeps potentially active transposable elements in a silent state (Chomet et al. 1987; Schwartz and Dennis, 1986) and if maintenance methylation of newly synthesized DNA strands preserves this epigenetic condition beyond mitoses, chromosome breaks with the potential formation of two repaired (and therefore unmethylated) DNA strands in the same molecule might create just the unmethylated condition necessary to start activity of a transposable element. In this case, the activation of silent transposable elements might be a chance event removing an epigenetic alteration and potentially leading to many real mutations thereafter, but not a special mechanism, by which the plant manages to escape 'stress'.

Again, we are in a difficulty. Interesting as the idea of the genomic stress is, we are still lacking experimental or observational confirmation. For all these reasons it will be necessary to do more experimental work in order to eventually understand better the basis for the retention of transposable elements in so many organisms.

**Evolution, role and control of transposable elements**

In the rest of my contribution I will briefly mention three problems.

*(1) How do transposable elements evolve?*

Active elements often are inactivated by internal deletion, which is discussed in the next section. In the *Ac* family, however, inactive, so-called *Ds* elements can also have more pronounced sequence alterations. These may either be a scrambling of existing sequences, as in the case of *Ds*2, or it can be the acquisition of sequences without any discernible homology to *Ac*, as is seen both in *Ds*1 and *Ds*2. These sequences may not be taken up by chance events. Both *Ds*1 and *Ds*2 differ from *Ac* or from ordinary *Ds* elements by their capability to be transposed by elements other than *Ac*. In the case of *Ds*1, the second active element is *Uq* (Caldwell and Peterson, 1992), in the case of *Ds*2 it is *Ac*2 (Rhoades and Dempsey, 1987, quoted in Fedoroff, 1989). While both *Uq* and *Ac*2 have not been cloned and sequenced, they do not move ordinary *Ds*. Thus, the possibility exists that some non-autonomous transposable elements have a modular structure, enabling them to respond to more than one autonomous element. If this could be proven, it would strongly point to an adaptive value of this double control, though it could not be said whether this advantage exists for the host or for the 'selfish' DNA.

Another aspect of the evolution of transposable elements is the possibility of its horizontal transfer between species. In *Drosophila* this has been made likely for the P-element. In plants, the only indication is the suspicious homology between *Ac*, *Tam*3 of *Antirrhinum*, and the *hobo* element of *Drosophila*. More will have to be learned, before these indications can be evaluated properly.

Once an element, e.g., a particular *Ds* element, has been formed, it can undergo an evolution of its own. One example is the formation of a 'double-*Ds*' from a particular deletion derivative of *Ac* by the insertion of this element into a copy of itself. This structure is responsible for the formation of *Ac*-induced chromosome breaks, a capability not found in *Ac* itself. From 'double-*Ds*', larger structures can be formed which are bordered at both ends by the *Ds*-element and which include long DNA stretches unrelated to the transposable element.

*(2) Can transposable elements serve as a probe for chromosome structure?*

The fact that transposable elements can be inserted in many positions of all chromosomes allows the question to which extent they behave differently, depending on their insertion site. The study of this question is still in its beginnings. It is known, however, that transposable element *Ac* can cause a different rate and timing of transposition, depending on its insertion site. The effect is exerted both on the element in *cis*, as on an independent *Ds*-element in *trans*. The latter makes it likely that it is the transcription of *Ac* which depends on the insertion site, but attempts to prove it have not yet been successful.

*(3) How is the transposition rate and the copy number of transposable elements kept low?*

At least in plants, it is usual that the number of inactive copies of a transposable element is large, often $>50$. Perhaps there is selection against too many active copies. The fact that active transposable elements can be silenced epigenetically by methylation supports this hypothesis. The observation that these frequent inactivations of transposable elements are confined to eukaryotes, and are not typical for bacteria, may be a consequence of the high transposition rate in plants when compared with the IS elements and transposons of bacteria. Perhaps there is no necessity in the latter to protect their genome against too frequent mutations caused by transposable elements.

One problem deserving attention is the rate of transposition. While this might not trouble the geneticist, who rather considers the mutations caused by transposable elements as frequent as compared to the usual base substitutions or frame-shifts from a genetic point of view, the

enzymatic reaction excising or transposing one of these elements is even rarer than DNA replication. While the latter occurs once during each cell cycle, transposable elements move with probabilities ranging from $10^{-3}$ to $10^{-2}$ in plants to $10^{-5}$ to $10^{-3}$ in bacteria.

In a number of transposable elements, a similar basic mechanism has evolved for the control of transposition rates. In several independent systems in bacteria and also in *Drosophila* the transposition is caused by one enzyme and it is inhibited by a second protein molecule derived from the same gene. The inhibitor of transposition is usually shorter than the transposase, but shares with it the DNA-binding domain. The short inhibitory molecule is usually found in higher concentration than the transposase itself. The ways in which this pair of molecules is produced, differ. Two promoters (Tn5), translational frameshifts (Is1 or retroviruses), or a splicing anomaly (P-element in *Drosophila*) have all been exploited to this end.

Transposable element *Ac*, however, does not seem to make use of two mRNA molecules. Only one RNA molecule has been detected. It is still possible that a shorter degradation product of the full-length protein that is found in maize endosperm is used as a transposition inhibitor. This protein is a minority component, however. It lacks its N-terminus and, according to its molecular weight, also the DNA-binding domain. If it were to exert an inhibitory effect on transposition, it would have to do so by binding to the active transposase protein, and as it is present only in a small minority, as compared to the full-length molecule, it would have to be able to inhibit a large number of intact protein monomers, possibly in an oligomeric state.

The question of oligomerization deserves further study. Kunze et al. (1993; and personal communication) have shown that in a transient assay in *Petunia* protoplasts, several mutants are capable to inhibit excision of transposable elements, even if these mutants produce a protein devoid of the DNA-binding site. This strongly indicates that the mutant protein is found in a complex that in its active form would be the transposase. M. Heinlein (personal communication), however, has detected large, rod-like structures by immunofluorescence. These cannot be described as oligomers, as they are much larger. They are found in maize nuclei. In transient expression experiments in *Petunia* protoplasts, they are also found. If the protein is made at much higher concentrations under the control of strong promoters, the number of these rod-like structures increases strongly. No increase in transposition frequency, however, can be observed. Analysing the experiments in detail, the hypothesis has been formulated that the rod-like structures, unlike the active oligomers, are something which removes transposase monomers from the active state. This might be a novel mechanism for keeping the transposition rate low and supports the finding by Becker et al. (1992) that even for the strongest promoters, the transposition rate quickly saturates and is never much higher than the transposition rate found when the transposase is made from an RNA synthesized under the control of the very weak *Ac* promoter. Further experiments are needed to support these notions. If they were borne out, they would be another example that evolution of transposable elements has used quite variable mechanisms in order to keep the transposition rate low.

At the moment, this is no more than a specialized interest of those trying to understand transposable elements, and particularly those who want to use transposon tagging as a gene isolation method and are therefore interested in controlling the rate of transposition and its tissue specificity. It is possible, however, that the study of these reactions will allow us to better understand biochemical reactions which are extremely rare. If very rare biochemical reactions occur during critical periods of the development of multicellular organisms, these studies may have a spin-off eventually.

In summary, however, it must be said that at present transposable elements are a specialty and that their importance for general biology is yet to be demonstrated.

## REFERENCES

*Most of the facts quoted here can be found and are referenced in the following reviews:*


Döring, H.P. and Starlinger, P.: Barbara McClintock's controlling elements: now at the DNA level. Cell 39 (1984) 253–259.

Döring, H.P. and Starlinger, P.: Molecular genetics of transposable elements in plants. Annu. Rev. Genet. 20 (1986) 175–200.

Fedoroff, N.V.: Maize transposable elements: a story in four parts. In: Fedoroff, N. and Botstein, D. (Eds.), The Dynamic Genome. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1992.

Gierl, A. and Saedler, H.: Plant-transposable elements and gene tagging. Plant Mol. Biol. 19 (1992) 39–49.

Lee, M. and Phillips, R.L.: The chromosomal basis of somaclonal variation. Annu. Rev. Plant Physiol. Plant Mol. Biol. 39 (1988) 413–137.

Nevers, P., Shepherd, N.A. and Saedler, H.: Plant transposable elements. Adv. Bot. Res. 12 (1986) 103–203.

Saedler, H. and Starlinger, P. Twenty-five years of transposable element research in Cologne. In: Fedoroff, N. and Botstein, D. (Eds.), The Dynamic Genome. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1992, pp. 243–263.


*A few papers not quoted in the above reviews:*


Becker, D., Lütticke, R, Li, M. and Starlinger, P.: Control of excision frequency of maize transposable element *Ds* in *Petunia* protoplasts. Proc. Natl. Acad. Sci. USA 89 (1992) 5552–5556.

Caldwell, E.E.O. and Peterson, P.A.: Transposable element systems in maize: interactions among components. Genetics 131 (1992) 723–731.

Keller, E.F.: A Feeling for the Organism. The Life and Work of Barbara McClintock. W.H. Freeman, New York, 1983.

Kunze, R., Behrens, U., Courage-Franzowiak, U., Feldmar, S., Kühn, S. and Lütticke, R.: Dominant transposition-deficient mutants of maize *Activator* (*Ac*) transposase. Proc. Natl. Acad. Sci. USA (1993) in press.

Lewin, R.: A naturalist of the genome. Science 222 (1983) 402–405.