A MECHANISM THAT COUPLES DNA TRANSPOSITION TO CELL DIFFERENTIATION (HYPOTHESIS)

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Rakowiecka 36; 02-532 Warszawa, Poland

A hypothesis underlying the significance of cytoplasmic events in the process of DNA transposition is proposed. To appear at a new chromosomal site, the transposon sequence is assumed to be first transcribed and then, in a form of the RNA copy, transported to the cytoplasm. A translation-dependent selection of RNA transcripts for reverse transcription is then made and the resulting single-stranded DNA copies return to the nucleus. The free copy may integrate within a new, transcriptionally inactive region of the chromosomal DNA. As a net result, cell differentiation advances in a manner coupled to the message utilization. The hypothesis finds support in many observations, particularly those made for plant cells.

Transposable genetic elements, first detected in maize (McClintock, 1956), appear now to be common for bacteria, fungi, higher plants and animals (Campbell, 1980). The mechanism of transposition has been, however, most extensively studied in prokaryotes, where the process has been found to be intrinsically coupled to DNA replication (Calos & Miller, 1980). While the same, probably, holds for eukaryotes a stage requiring the participation of a reverse transcriptase activity appears to be necessary for transposition to occur in eukaryotic cells (Sharp, 1983). Striking similarities between movable genetic elements and retroviral proviruses (Temin, 1980) strongly suggest that cellular RNA may be an essential intermediate on the way by which a transposon appears at a new chromosomal site. The viral genome is, however, reversely transcribed in cytoplasmic cell compartments, whereas the DNA transposition is generally considered as a typical nuclear event. Here, we propose a hypothesis which emphasizes the significance of cytoplasmic processes for DNA transposition in differentiating eukaryotic cells.

* The views presented in this section by the authors might be not fully endorsed by the editors.

[193]
The model

The hypothesis proposed requires a transposon sequence to be transcribed by a nuclear RNA polymerase as the first stage of the transposition process. The resulting transcript is assumed to be transported to the cytoplasm where it serves as a template for DNA synthesis. This newly-synthesized extrachromosomal DNA is then transported to the cell nucleus to be integrated into the nuclear genome in a way similar to that known for proviral DNA integration. While, however, the proviral DNA integration leads to the cell transformation, the integration of the cellular RNA-derived DNA will lead to the normal cell differentiation. The overall mechanism is summarized in Fig. 1.

![Diagram]

Fig. 1. A schematic representation of the postulated mechanism of eukaryotic DNA transposition. 1, Transcription of a transposon sequence and transportation of the resulting transcript from the nucleus to the cytoplasm. 2, Reverse transcription of the transposon RNA copy. 3, Transportation of the single-stranded copy (s-s DNA) of the transposon from the cytoplasm to the nucleus. 4, Replication-coupled integration of the free transposon copy into the chromosomal DNA. With respect to the DNA sequence organization, one of the two progeny DNA molecules does not differ from the parental DNA, whereas the other one (rearranged) differs from it. For details of the stage 4, see Fig. 2.

Evidence to suggest this hypothesis has come mainly from studies on DNA synthesis in plant cells. The first step of the proposed sequence of events (see Fig. 1) does not need a special justification. Eukaryotic transposons are known to be extensively transcribed and their RNA copies have been found among cellular RNA species (Shiba & Saigo, 1983). The crucial and, probably, most controversial point is the possibility of non-organellar DNA synthesis in the cytoplasm. Although microsome-associated DNA was first described twenty years ago (Bach, 1962) and has since been found in a wide variety of eukaryotic cells (Reid & Charlson, 1979), its origin and physiological significance still remain unclear. According to the prevailing view, it represents mainly, if not exclusively, artefactual products of nuclear DNA degradation. Contrary to this view, however, in germinating wheat embryos, cytoplasmic non-organellar DNA has been found to appear from de novo synthesis at an early germination stage, prior to the initiation of the chromosomal DNA replication (Buchowicz et al., 1978). It has been concluded that the cytoplasmic DNA appears from reverse transcription of those RNA messages which reach the cytoplasm but are not used for translation. There is, however, no direct evidence for the involvement of reverse transcriptase in the synthesis of the cytoplasmic DNA. Nevertheless, its single-strandedness (Kraszewska & Buchowicz, 1980) is consistent
with such an assumption. Moreover, this assumption remains in accordance with the recently accumulating data on the origin of processed genes and dispersed repetitive DNA sequences in other eukaryotic cells (Sharp, 1983). With respect to its size (about 6000 nucleotide residues), the wheat embryo extrachromosomal DNA (Kraszewska & Buchowicz, 1983) corresponds to free copies of typical eukaryotic transposons (Flavell & Ish-Horowicz, 1981).

The next two steps, transportation of DNA from the cytoplasm to the nucleus and its integration into the nuclear genome, are included into the scheme for the following reasons. (1) Fungal plasmids are known to move from the cytoplasm to the nucleus and to integrate within the nuclear genome (Falco et al., 1982; Wright & Cummings, 1983). (2) For germinating wheat embryos, a precursor-product type relationship between cytoplasmic and nuclear DNA has been demonstrated (Buchowicz et al., 1978). And, (3) isolated wheat embryo cell nuclei have been found to take up the newly-synthesized single-stranded cytoplasmic DNA and to integrate it into heavy nuclear structures (Kraszewska & Buchowicz, 1983). Other DNA fragments, although taken up readily, remained either unchanged or underwent a rapid degradation within the nuclei. Thus, the ability to integrate seems to be a property of the cytoplasmic DNA. The property is typical for DNA species (or DNA fragments) bearing insertion sequences such as those occurring in transposons and proviruses. It is, however, generally accepted that a donor molecule for either transposition or integration should be in a double-stranded form. Nevertheless, an alternative view may be proposed from the recent findings of Krimer & Van’t Hof (1983). These authors have found branched DNA molecules in pea root-tip cells. The branched forms are linear, partially double-stranded, with single-stranded branches of a size corresponding roughly to that of the single-stranded cytoplasmic wheat embryo DNA. It is quite conceivable that such structures may represent early intermediates in the process of the integration of single-stranded extrachromosomal DNA into chromosomal DNA.

The integration may proceed with a transient formation of branched forms as shown in Fig. 2. A preliminary step required for the integration to occur is the

![Fig. 2. A sequence of events leading to the integration of a free transposon copy into chromosomal DNA. The thin lines represent the strands of a nuclear DNA molecule fragment and the replication products made on them. The thick lines correspond to the cytoplasm-derived single-stranded transposon copy and the complementary strand made on it during integration. 1, Gap formation. 2, Association and 3, ligation of the transposon copy to the gapped DNA. 4, Conversion of the branched structure into a replicative fork. 5, Movement of the replication fork. 6, Appearance of the replication products one of which bears (on both strands) the inserted transposon copy.](image-url)
formation of a gap within the acceptor molecule. Gaps of various size are known to be formed spontaneously in genomic DNA (Wortzman & Baker, 1981). In the case of seed DNA, a particularly large number of gaps may accumulate during the storage period. Each of them may serve as a potential acceptor site for the linear single-stranded DNA molecules after germination starts. The only prerequisite for the initiation of the gap filling will be the base complementarity between the donor and acceptor DNA strands. The association products will be suitable for ligation which would complete the branched structure formation. Such a structure resembles replication forks and may be easily converted into a typical replication fork by synthesis of the strand complementary to that associated with the gapped DNA. Once formed, the replication fork can move till the replicon end. As a result, two non-identical progeny DNA molecules (or DNA fragments) may be formed. One of them will be obviously the same as the parent DNA, whereas the other will contain the inserted sequence. The insert, although not foreign to the genome, appears in a new chromosomal location as it is required to complete the transposition process.

**Implications**

The model proposed by us differs essentially from those described earlier. Instead of the replicon fusion (Isberg & Syvanen, 1981; Reed, 1981), roll-in replication (Harshey et al., 1982) or illegitimate recombination events (Schierer & Davis, 1980; Roeder & Fink, 1982; Berg, 1983), a transposon copy-induced formation of replication fork is postulated. The most important difference concerns, however, the dependence of DNA transposition on cytoplasmic events. After reaching the cytoplasm, transposon transcripts are either utilized for the cell translational needs or serve as templates for reverse transcription. Thus, the transposition and resulting DNA sequence rearrangement are coupled to protein biosynthesis. Such a coupling may create a mechanism controlling the cell differentiation process. "Unnecessary" (not utilized for translation) messages may be eliminated from further expression if their DNA copies are inserted into transcriptionally inactive chromosome regions. As a result, the progeny cells would become more specialized in protein production. In finally differentiated cells, no DNA copy of the transposons may be expected to appear in the cytoplasm. In full accordance with this expectation, cytoplasmic nonmitochondrial DNA is known to occur in embryonic and differentiating cells but not in differentiated ones (Koch, 1973). In connection with this, unintegrated proviral DNA of retroviruses may be considered as the viral counterpart of cell cytoplasmic non-mitochondrial DNA. The unintegrated proviral DNA can, probably, mimic the cellular non-mitochondrial DNA on its way from the cytoplasm to the nuclear integration sites. Since, however, proviral DNA carries a foreign information, its integration into the host genome will lead to the cell transformation instead to allow the normal cell differentiation.

The apparent occurrence of transposition events in monocotyledonous plants which are never infected with either retroviruses or DNA viruses, argues against
the recently favoured view that movable genetic elements evolved from retroviruses (Shiba & Saigo, 1983). It may rather be that the existence of eukaryotic transposons and their involvement in the cell differentiation process have made it possible for retroviruses to appear in evolution.

**Conclusion**

It may be inferred from recently published experimental data that movable genetic elements are involved in the cell differentiation process by a mechanism that requires their transcription, appearance of the RNA copies in the cytoplasm, reverse transcription and integration of the single-stranded DNA copies into new chromosomal sites. Branched, fork-like "replication" structures, found in plant cells, are proposed to represent early intermediates of the integration stage. Replication initiated at such structures may lead to the appearance of products that differ from each other with respect to the DNA sequence organization (unchanged and rearranged, respectively). A substitution of foreign DNA (e.g., proviral) for a cellular transposon copy may result in the cell transformation, instead of leading to the normal cell differentiation.

**REFERENCES**


MECHANIZM WIAŻĄCY PRZEMIESZCZANIE SIĘ DNA Z RÓŻNICOWANIEM KOMÓREK (HIPOTEZA)

Streszczenie

Praca niniejsza opisuje hipotezę postulującą udział wydarzeń cytoplazmatycznych w procesie przemieszczania się (transpozycji) DNA w komórce eukariotycznej. W myśl proponowanej hipotezy, pierwszym etapem transpozycji jest transkrypcja sekwencji przemieszczających się. Produkt transkrypcji jest następnie transportowany do cytoplazmy, gdzie, w zależności od potrzeb translacyjnych komórki, jest albo wykorzystywany w biosyntezie białka, albo też służy jako matryca do odtworzenia, drogą odwrotnej transkrypcji, jednoniciowej kopii transpozonu. Kopia ta wędruje napowrót do jądra komórkowego, gdzie następuje jej integracja w nowym, transkrypcyjnie nieczynnym rejonie chromosomalnego DNA. Przemieszczony w ten sposób odcinek DNA jest wyłączony z dalszej ekspresji, co prowadzi do charakterystycznego dla procesu różnicowania się postępu w zakresie specjalizacji komórek w produkcji białka. Wysunięta hipoteza opiera się na licznych obserwacjach i dowodach pośrednich, pochodzących zwłaszcza z badań nad pozachromosomalnym DNA komórek roślinnych.

Received 11 July, 1983