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Nucleo-kinetoplast interactions and variability in the kinetoplastida

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Summary

A detailed electron microscope study of epimastigotes of some *Trypanosoma cruzi* strains and of *Leishmania* spp. promastigotes has revealed in these not only a close spatial proximity between the nucleus and kinetoplast but also a direct contact between these two compartments and even their occasional tight joining. These contacts are seen to evolve in parallel with trypanosomatid development, even within the limits of one stage of the life cycle, and to become more obvious during the parasitic cell differentiation into invasive form. A possibility of exchange between genetically important molecules at the contact of two different DNA-containing cell structures is discussed in terms of the role of kinetoplast DNA minicircles as a source of genotypic diversity and transposable genetic elements. Moreover, these events are regarded as being presumably involved in the specific mechanism of variability characteristic of the agamic protozoa of the order Kinetoplastida.

Key words: Kinetoplastida, *Trypanosoma, Leishmania*, nucleus, kinetoplast, nucleo-kinetoplast interactions, genetic variability

Introduction

The variation and heredity of agamic protozoa still remains poorly understood. The kinetoplastid flagellates make one of the largest groups of agamic protists. So far no attributes of the sexual process (karyogamy or meiosis) have been detected despite extensive study with the most up-to-date techniques. Meanwhile, recent studies into DNA, isoenzymes, antibodies or some other biological features of trypanosomatids strongly testify to the presence of certain genetic recombinations in these flagellates (Tait, 1983; Tibayrenc et al., 1984; Tibayrenc and Ayala, 1991; Stuart and Feagin, 1992; Majumder, 1996).

On summarizing all these data, it is possible to state that genetic variation in trypanosomatids may occur even within the frames of individual clones. This variation may be accomplished very quickly, which poorly agrees with the classic rules of Mendelian heredity. Nevertheless, some few authors admit the existence of quite different genetic mechanisms operating in the kinetoplastids (Poljansky, 1982; Gibson et al., 1985; Baker, 1989).

It should be emphasized that the genetic situation in the kinetoplastid cells is very peculiar, because along with the nucleus they harbor another functioning DNA-containing structure termed the kinetoplast. This is a highly organized nucleoid of the huge single mitochondrion of the kinetoplastids, containing up to 25% of the whole DNA in the cell, called kinetoplast DNA or kDNA. Its presence seems to provide the kinetoplastids with some distinctive opportunity of genetic combinations to be eventually realized not only between cells-individuals, but also between different compartments within the same cell, and also presumably at the molecular level (Kallinikova, 1987).

The present paper deals with our recent studies of space interactions between two different DNA-containing cellular compartments in representatives of the Kinetoplastida – some trypanosomatid pathogens causing disease in humans and animals.

Material and Methods

Five *Trypanosoma cruzi* strains were grown in culture using peptone-glucose and peptone-maltose nutrition media. Some *Leishmania* species (*L. major, L. donovani, L. infantum, L. gymnodactyli*) were cultured on classic biphasic NNN medium.

Within one passage, the harvested flagellates were taken from the culture every 3 or 4 days, smeared, dried, fixed in ethanol and stained with Romanovsky-Giemsa counterstain. The pictures of relative nucleus-kinetoplast positioning were compared in parallel with the patterns of

cell growth in culture, general cell morphology and mode of cell transformation. In all, several tens of replicates of similar observations were made.

T. cruzi flagellates of one strain, grown in culture on NNN medium, were examined with electron microscope 21, 23, and 35 days after passage. The flagellates were purified from the medium by pelleting the cells at 2,000 rpm for 10 min, washed twice in isotonic NaCl solution, fixed with 1.5.% glutaraldehyde in 0.1M cacodylate buffer (pH 7.2–7.4) at 4°C for 2 hours, postfixed with 2% OsO_4 in the some buffer and under the same time and conditions.

After dehydration in a series of alcohols and anhydrous acetone the cells were embedded in Epon 812 to be polymerized at 60°C in 24 h. Sections were stained with uranyl acetate and lead citrate and viewed with a JEM-100B transmission electron microscope.

Results

It is commonly known that only the invertebrate part of the trypanosomatid development can be reproduced on artificial nutrition media. In nature the part of the life cycle proceeding in the invertebrate host involves epimastigotes in trypanosomes and promastigotes in leishmanias.

A characteristic feature of the epimastigotes is a close relative position of their nucleus and kinetoplast in the anterior third of the cell. In the majority of *T. cruzi* forms both these structures were disposed more or less close to each other, but looked as two separate discrete formations (Fig. 1*A*). In other epimastigotes the kinetoplast was much more displaced towards the posterior end of the cell, just at the level of the nucleus and a bit alongside of it. In these epimastigotes the kinetoplast still remained discrete and kept its oval shape (Fig. 1*B*). Cells of this type along with

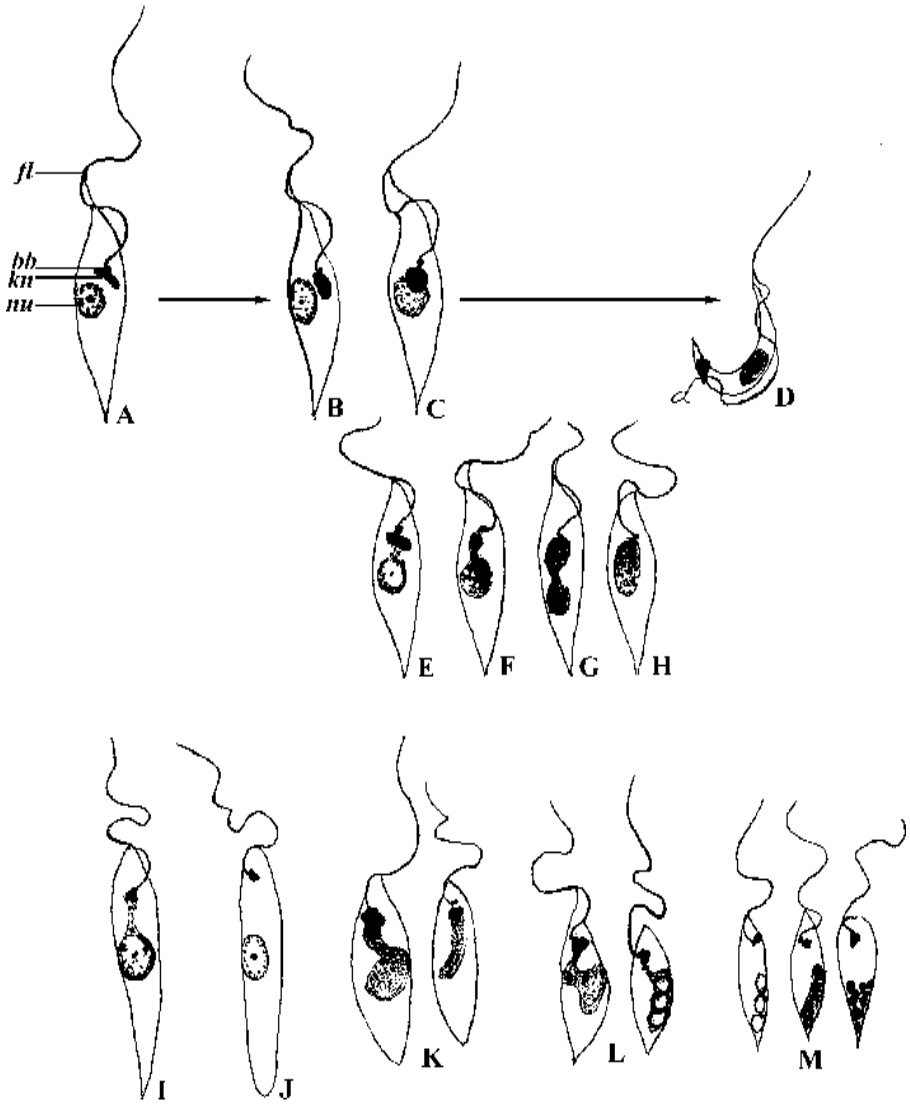

Fig. 1. Patterns of the nucleo-kinetoplast interaction in the process of differentiation of the epimastigote and promastigote forms in some kinetoplastids. A–*C*, *E–H* – *Trypanosoma cruzi*, epimastigote forms, *D* – *T. cruzi*, metacyclic form; *I–M* – *Leishmania*, promastigote forms, *I* – lightening, *J* – light, *K*, *L* – transitional, *M* – "metacyclic" forms.
bb – basal body, *fl* – flagellum, *kn* – kinetoplast, *nu* – nucleus.

other cells, in which the rounded kinetoplast was located above the nucleus (Fig. 1*E*), reflected the process of epimastigote transformation to metacyclic forms with their characteristic round-shaped kinetoplast occupying the posterior position in the cell (Fig. 1*D*).

Along with the above cells, some other cells were available in our material, in which the nucleus and the kinetoplast were seen as being in much closer vicinity of each other, rather than as two neighboring structures. In some epimastigotes these were either connected via a cross-piece, no peripheral nuclear chromatin being seen in the point of contact (Fig. 1*E*), or represented a common evenly stained dumb-bell- or oval-shaped structure (Figs 1*F–H*). Similar pictures were not rare and could be readily observed in all five investigated *T. cruzi* strains grown on the used nutrition media.

In the promastigote forms of trypanosomatids the nucleus and the kinetoplast are commonly separated in space, so that the former is situated in the center or in the anterior third of the cell, while the latter is located in its anterior end. In our material, at least three different types of spatial arrangement between the nucleus and kinetoplast could be recognized. 1. Their well-defined spatial separation without any obvious contact in-between, which well compares with the situation in the conventional promastigotes (Fig. 1*J*). 2. Their obvious association via a narrow cytoplasmic band shown to be rich in RNA (Fig. 1*I*). 3. An extended direct contact between these two, looking like a broad nuclear "sleeve" stretched towards the kinetoplast (Fig. 1*K*), or resembling a cross-piece between them (Fig. 1*L*). The eventual join (fusion) of the two structures results in the formation of a common large body. Its Feulgen staining looked homogenous, with somewhat stronger color in the part corresponding to the kinetoplast location. Observations of similar nucleo-kinetoplast contacts were not single and were noticed in all examined *Leishmania* species.

Electron microscope investigation of *T. cruzi* epimastigotes has shown that the nucleo-kinetoplast contacts may be very tight indeed, but in spite of this the partners' membranes, in most cases, remain intact (Fig. 2*A*). Rather often, however, the membranes in the contact area looked folded or obscure, with the enlarged intermembranous spaces in both structures. Occasionally, a nuclear pore (not seen on the picture) was recognized in this area along with a chromatinous "glade" inside the nucleus (Figs 2*B–E*). Sometimes a direct join between the nucleus and kinetoplast was accompanied with an obvious fusion of their membranes (Fig. 2*F*). The part of the kinetoplast free from the kDNA network was involved in this process.

The nucleo-kinetoplast interaction in *T. cruzi* regularly changed within the time of one passage, thus reflecting the parasites' development in culture. The progressive movement of the structures to each other was seen to achieve eventually its maximum as the culture passed to the stationary growth phase (Fig. 3), when these contacts were most numerous and their images most convincing. No wonder that the increased frequency of the contacts directly correlated with metacyclogenesis in the epimastigotes. Both processes displayed similar dynamics within the same passage, and the maximum frequency of the observed contacts preceded metacyclogenesis. The closest nucleo-kinetoplast interaction occurred in the *T. cruzi* strains with more active metacyclogenesis and on the growth media providing the most active metacyclogenesis (Fig. 3). However, the closest contacts between these organelles are not always accompanied with the adequate metacyclogenesis activity.

The spatial relations between the nucleus and kinetoplast in *Leishmania* promastigotes well compare with their dynamic development in culture. As shown elsewhere (Kallinikova et al., 1987), *Leishmania* promastigotes undergo regular differentiation in culture, accompanied by a decrease in their basophilia (fading of the cytoplasm), and by changes in form and dimensions of cells, and in the structure and arrangement of cell organelles. In particular, the separation between the nucleus and kinetoplast was noticed to increase little by little. However, in *Leishmania* promastigotes, in contrast to *T. cruzi* epimastigotes, it is the nucleus, rather that the kinetoplast, that passes eventually to the posterior part of the cell.

The two above fundamentally different forms of contacts between the nucleus and kinetoplast were timed to some definite and distinct steps of differentiation in *Leishmania*. The phenomenon of promastigote fading after the active division of dark forms was accompanied by reapprochement of these intracellular structures. On moving away from the kinetoplast to its central position, the nucleus left behind a cytoplasmic "train" rich in RNA, looking like a bridge spanning both these structures (Fig. 1*I*).

The nucleo-kinetoplast integration into one common body was characteristic of another developmental step – conversion of promastigotes into the final small light forms, – which coincided with most significant structural changes in the promastigote nucleus. Pale and homogenous nuclei (Fig. 1*K*) or nuclei with circular chromatin structures (Fig. 1*L*) were seen in intimate contacts with the corresponding kinetoplasts; the latter nuclei, not yet having reached the posterior position, are characteristic of the onset of leishmanian metacyclogenesis. The nuclei of the final metacyclic promastigotes displayed a homogenous, blister or granular structure and totally occupied the terminal pointed end of the cell (Fig. 1*M*). At this final step any direct contacts between the nucleus and kinetoplast are rare.

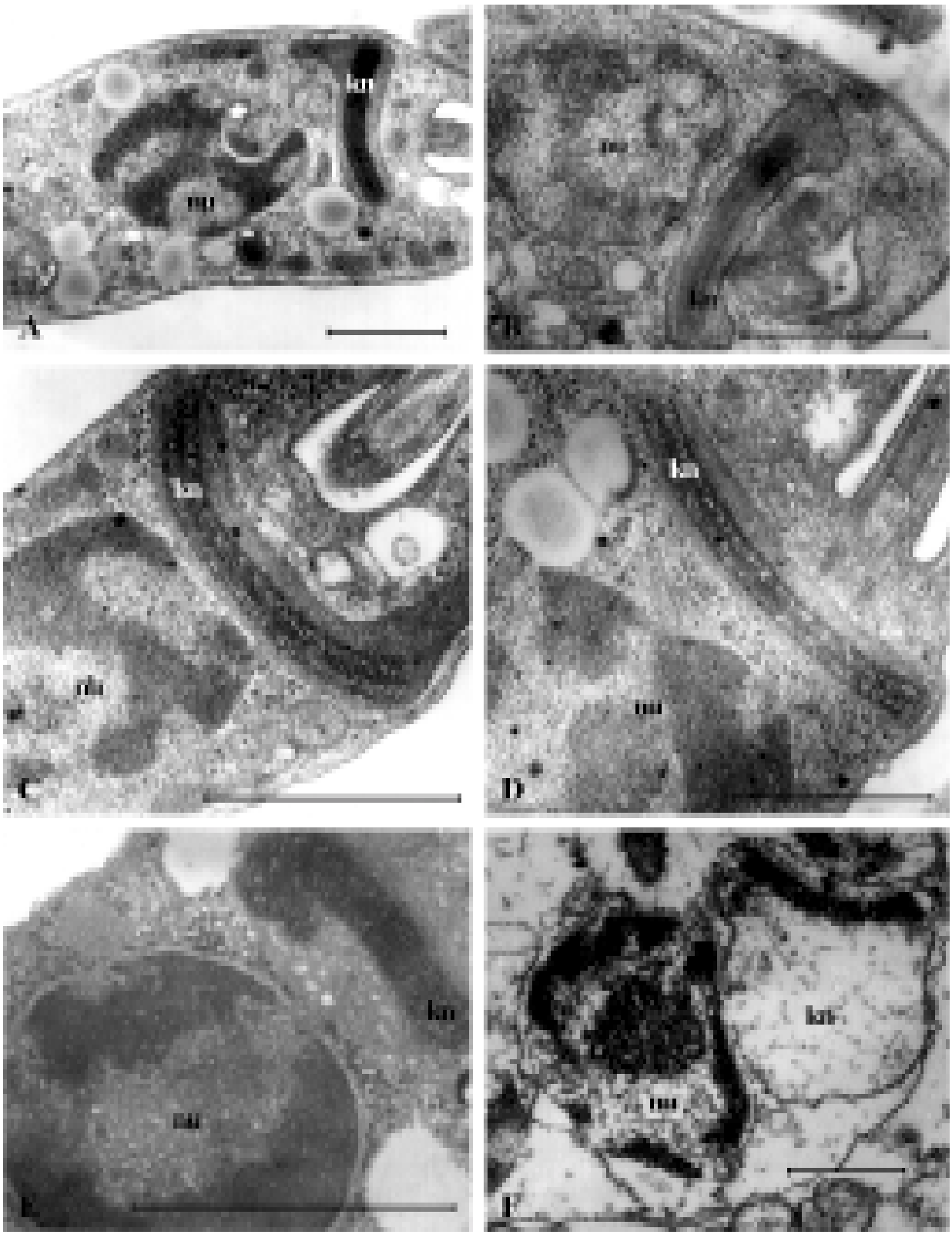

Fig 2. The ultrastructure of the contacts between the nucleus and kinetoplast in *Trypanosoma cruzi*. *A* – the nucleus and kinetoplast are distinctly separated; *B* – their interaction is indefinite; *C–E* – a nuclear pore directed towards the kinetplast; *F* – membranes of the nucleus and kinetoplast look united. *Scale bars:* 1 μm throughout.
kn – kinetoplast, *nu* – nucleus.

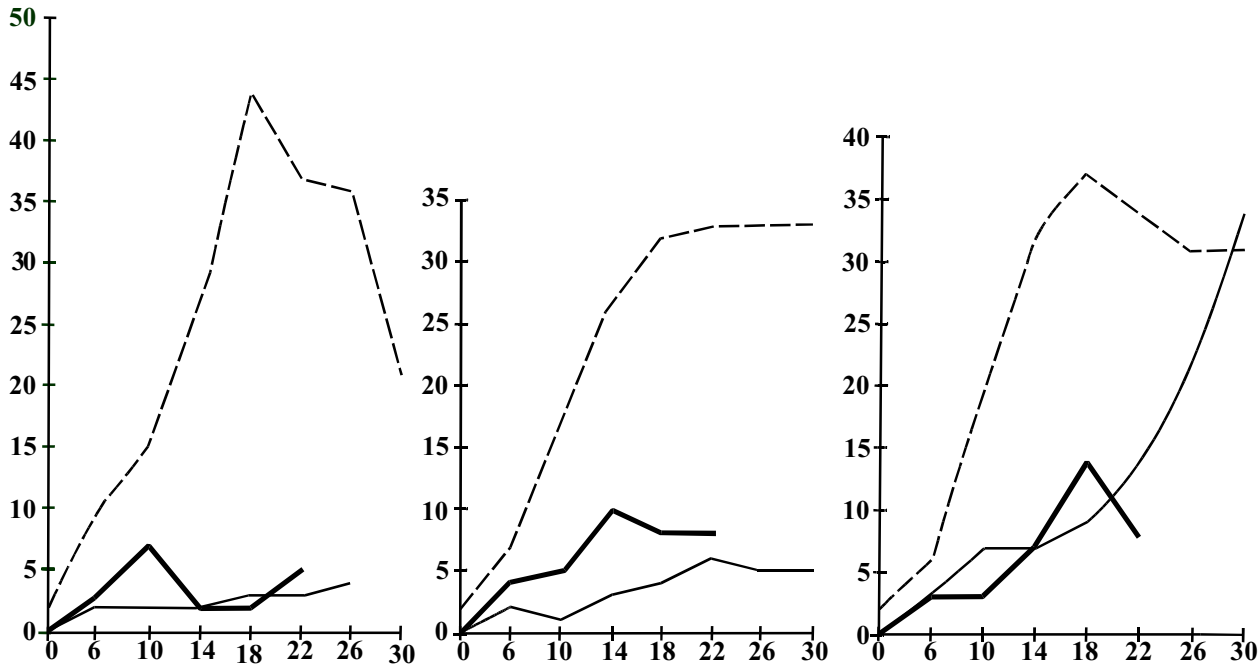

Fig. 3. *Trypanosoma cruzi*: growth, metacyclogenesis and contacts of the nucleus and kinetoplast in cultural passage. *A* – a strain unable to metacyclogenesis, *B* – strains with a weak metacyclogenesis, *C* – strains with an active metacyclogenesis. *abscisse* – days after inoculation, *ordinate* – the number of cells or percentage of different forms, *dotted lines* – the number of cells (one grade means 2 million of cells in 1 ml), *thin line* – percentage of metacyclic forms in population, *thick line* – percentage of epimastigote forms that display direct contacts between the nucleus and kinetoplast.

Discussion

The changes in the nucleo-kinetoplast interaction constitute the key event of cell differentiation in the trypanosomatid flagellates. This permits to break up their life cycle into some distinctly outlined stages characterized, in addition, with some specific morphotype and different levels of organization of the flagellar apparatus.

The epimastigote stage, wherein the nucleus and kinetoplast have been observed to approach one another, is very important for the normal development to proceed. It is well known that a prolonged elimination of this stage from circulation, e.g. upon maintenance of the parasite in a vertebrate host only, invariably results in a loss by trypanosomes of their ability to accomplish the whole life cycle.

As has been demonstrated in the present paper, the approximation of the nucleus and kinetoplast can occur also at some other stages of the trypanosomatid life cycle, in particular, at the promastigote stage of *Leishmania*. Similar light microscopic observations of the *Leishmania* kinetoplast overlapping its nucleus were reported by Christophers et al. (1926). Numerous confirmations of this phenomenon can be found in some later and recent publications dealing with electron microscopy of epimastigotes in various *Trypanosoma* species: *T. cruzi* (Clark and Wallace, 1969, Fig. 13; Schulz and MacClure, 1961, Figs 1, 3; Inoki et al., 1971, Figs 6, 9–11, 14; Inoki et al., 1973, Figs 2, 4*), T. brucei* (Steiger, 1973, Figs 40, 41, 43, 44*),* and also in some other trypanosomatid genera – *Leptomonas, Herpetomonas, Crithidia, Blastocrithidia, Phytomonas, Leishmania, Endotrypanum*. In many cases the nucleo-kinetoplast contacts were detected at those stages for which the constant close relative disposition of these was not typical (Chacraborty and Sanyal, 1962; Mühlpfordt, 1963, 1964; Molyneux et al., 1975; Podlipaev, 1985; Skarlato, 1987; Podlipaev and Frolov, 1999). This was also reported for the bodonids – another kinetoplastid group (Mylnikov, 1986a, Fig. 14; 1986b, Fig. 1c; Frolov et al., 1996; Skarlato and Lom, 1997, Fig. 2). It is a pity that the research community studying the kinetoplastids does seem to deserve attention to this phenomenon. Although, the peculiar nucleo-kinetoplast interaction appears to be the commonest event in the kinetoplastid life history, being indispensable at the epimastigote stage but not so regular at the rest stages.

We have succeeded to demonstrate that such a contact coincides with a particular step in the parasite's development even within one and the same stage. In our material this contact was coincident with the appearance of the classic metacyclic forms in *T. cruzi* and in the final forms of *Leishmania* transformation in culture, which referred to as metacyclic forms (Kallinikova, 1987) and which appeared truly invasive (Kallinikova et al., 1992).

The virulence of *Leishmania* forms directly correlated with the picture of nucleo-kinetoplast contacts (Greenblatt et al., 1985).

Thus, the availability of nucleo-kinetoplast contacts in the kinetoplastid flagellates may be a much more significant phenomenon than a mere transformation to metacyclic forms. These contacts are obviously involved in the establishment of invasive forms, i.e. the forms with potencies to continue the life cycle in the vertebrate host.

It is important that the degree of direct contacts between different DNA-containing cellular compartments may be extended up to their immediate proximity. In *Leishmania* promastigotes examined with the light microscope it resulted in formation of a large evenly stained common body.

Analysis of the relevant electron microscope evidence indicates that the extent of the nucleo-kinetoplast unity remains rather indefinite in most part of cells of different kinetoplastids, which is in accordance with our own observations. In this concern, some presumable principles of the contact may be as follows. If the membranes of either partner look fuzzy, interrupted or confluent, but involving in the contact the DNA free spaces from both sides (perinuclear space free from nuclear chromatin and mitochondrial intermembranous space free from kDNA network, respectively) is obvious.

Mühlpfordt (1963, 1964) provided a most convincing evidence of a direct nucleo-kinetoplast contact, with the rupture of respective membranes over a considerable extension, thus making possible the exchange of the partners' contents. As in our case, Mühlpfordt observed this phenomenon in different trypanosomatids and at different stages of their life cycle, but for a small part of the population. It is very likely that such a direct contact between the nucleus and kinetoplast may be of short duration. But nevertheless this contact makes sense in terms of the genetic combination, i. e. it may provide exchange of genetically significant molecules between the partners within the cell.

Despite the fact of an obvious compartmentalization within the eukaryotic cell, a bulk of recent evidence suggests that there are no absolute barriers to genetic exchange between different DNA-containing cell compartments, although such exchanges are far from regular. Certain data are available on the gene migration from mitochondria to the nucleus; besides, some homology of nucleotide sequence was demonstrated in the nuclear and mitochondrial DNAs, respectively, in a wide diversity of subjects (Kung et al., 1972; Kemble et al., 1983; Levin, 1984; Jaffe and Schatz, 1984; Nagley and Devenish, 1989; Grivell, 1989; Thorsness and Thomas, 1990). One of the possible mechanisms of intermitochondrial gene exchange is a junction of these organelles. Generally speaking, such an intracellular genetic combination could be of particular probability and importance in the agamic unicellular organisms, in which this may serve as a molecular equivalent of the lacking sexual process. In particular, such a combination is of great significance for the Kinetoplastida due to the availability in these of the unique kinetoplast genetic system.

Bray (1973) presumed a leading role of recombination between the nucleus and kinetoplast in the course of the kinetoplastid evolution. The kinetoplast and its DNA have attached a great importance in parasexual interactions between individual kinetoplastid cells (Dean and Milder, 1966, 1972; Gibson, 1995). But both the nuclear and kinetoplast genetic systems can interact even within the scope of a single cell. As shown in this paper, the observed migration of the nucleus and kinetoplast within the same cell and their close interactions are accompanied with changes in their size, shape and structure.

The supposed genetic interaction between the two discussed compartments is supported by synchrony in their synthetic processes, by positive correlation of the nucleotide composition and the degree of variability from species to species, and, at last, by a partial homology of the nuclear and kinetoplast DNA sequences (Simpson and Silva, 1971; Hill and Bonilla, 1974; Steinert et al., 1976; Borst et al., 1982). Such a genotypic combination within a single cell can create some mechanism of widely reported intraclonal variability in the kinetoplastids (Hirumi et al., 1980; Goldberg and Pereira, 1981; Hoeijmakers and Borst, 1982; Wagner and So, 1990; Alves et al., 1996).

Some particular features of the two genetic systems can promote genetic exchange between the nucleus and kinetoplast. In the nucleus these features involve the peripheral disposition of chromatin, just under the nuclear membrane, and its permanent partly condensed state, and the presence of open reading frames in the nuclear DNA (Borst et al., 1982). In the kinetoplast, special attention is certainly drawn to kDNA, primarily to its minicircular molecules, making up 90–95% of this DNA.

The most characteristic features of minicircles are their small size and being present at a high copy number, tendency to catenation, ability to interact with other molecules, availability of a bent helical region in their DNA favoring integration with other DNAs, inactive, furtive or very specific transcription, extraordinary heterogeneity in sequence (from a few to hundreds of classes in one kinetoplast), and a surprisingly fast evolution. All these and other features of minicircles make it possible to regard these kDNA molecules as presumable transposable genetic elements. The earlier reported similarity between kDNA minicircles and the mobile genetic elements (Kallinikova, 1987) was then recognized also in some other, finer properties of the latter (Majumder, 1996).

A great variety of rearrangements in kDNA minicircles (e.g. insertions, or translocations) are thought to be involved in the process of kinetoplastid intraclonal variability, in the accomplishment of the life cycle (Morel et al., 1980), and even in genetic relations of the parasite with its host-cell (Texeira et al., 1994). The degree of minicircle heterogeneity for nucleotide sequences corre-

lates with certain phenotypic characters of the Kinetoplastida, such as the surface antigens and their variability, and with the taxonomy, phylogeny and evolutionary potencies of species and strains.

In seems hardly possible that such a unique reserve of the vast genetic diversity of these molecules, with the properties of transposable genetic elements, may be only an accidental occurrence in these agamic unicellular organisms. One of presumable functions of the "enigmatic" minicircles may be the creation, accumulation and realization of genotypic diversity and variability. Since the degree of variability is maintained by species (Tibayrenc et al., 1986) and the insertions are site specific, the intracellular combinations of the nuclear and kinetoplast DNAs can be channeled to a certain degree.

Our studies on *Leishmania* species enabled us to assume that the contact of the nucleus and kinetoplast may involve also an exchange of their RNAs. This assumption well compares with some data on other subjects and dealing with the import into mitochondria of small RNA coded by the nuclear genome (Martin et al., 1979; Nagley, 1989). Possible mechanism of this exchange may be associated with a recently discovered kinetoplastid capability of editing DNA transcripts with the involvement of kDNA minicircles, in particular with the involvement of guide RNAs synthesized on them.. It is important that this was observed just after cessation of promastigote active division due to their transition to differentiation, whose onset coincided with the spatial isolation of the nucleus and kinetoplast and with the translocation of the latter to the posterior position.

Thus, in thus agamic flagellates (Kinetoplastida), the contact between the nucleus and kinetoplast is possible at different stages of their life cycle. This contact is commonly timed to some particular developmental stage and is associated with acquisition of their ability to complete the life cycle. A direct communication of the nucleus and kinetoplast provides a real opportunity to combine their respective DNAs. In this process the genotypic diversity of kDNA minicircles can be realized.

Based of the potencies of the cell and on its reserves of genotypic diversity, a certain mechanism, operating only within some part of a population and being not very regular, may nevertheless account for the fast rate of kinetoplastid variability and genetic variation at the intraclonal level, and may set a certain degree of its channeling. And it is clear that this variability does not fit the postulates of the classic genetics.

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