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DIFFERENTIATION IN THE LIFE CYCLE OF TRYPANOSOMES

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SUMMARY

Differentiation processes in the trypanosomes life cycle are discussed on the basis of the recently proposed regulatory mechanisms for gene expression in bacteria and viruses.

INTRODUCTION

A fundamental feature of cellular differentiation is the development of specialized morphological and biochemical structures which accomplish specific functions. The process of cellular differentiation and the final activity of the differentiated cell require a very precise sequence of metabolic processes, all of which reflect a plan previously determined at the genetic level. In each phase of cellular life particular genetic information, appropriate to that phase, must have a predominant expression. The basic problem of differentiation is to understand the regulatory mechanism that allows predominant expression of appropriate information out of the totality of information contained in the genome.

The important progress recently made in the understanding of regulatory circuits in bacteria and viruses, especially the general model proposed by JACOB & MONOD [7] (Fig. 1) for the regulation of gene activity, opened a new approach to the study of differentiation. As pointed out by MONOD & JACOB [10], the application of these new concepts shows quite clearly that "biochemical differentiation (reversible or not) does not constitute a paradox as it appeared to do for many years to both embryologists and geneticists".

Some Protozoa may be suitable as tools in the study of cellular differentiation and this new approach may be useful in the understanding of many obscure aspects of the life cycle of Protozoa.

We find in Protozoa two favorable conditions for the study of cellular differentiation: the unicellular state and the cyclic reversibility of the differentiation process. The search of inducing repressing substances is simplified and the analysis of regulatory mechanisms avoids the intercellular relationship, hormone action and irreversibility observed in the differentiation process of most multicellular organisms. Thus, Protozoa may provide a bridge between the molecular and the organ levels in the study of differentiation.

Following a proposal of LWOFF & LWOFF [9] that cyclic events in Protozoa and trypanosomes may be interpreted on the basis of the recently developed regulatory mechanisms in bacteria and bacteriophage, we consider in more detail the application of such principles to a number of phenomena in the trypanosome life cycle.

Differentiation in the life cycles

Most trypanosomes show at least two phases of differentiation in their life cycle in relation to the change of the host and the multiplication process. After reproduction in the crithidial stage in the digestive tract of

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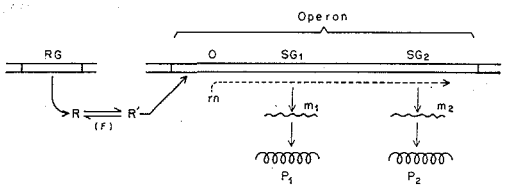

Fig. 1 — *General model of the regulation of gene expression* (from JACOB & MONOD [7]) SG_1 and SG_2 are structural genes, m_1 and m_2 their respective messengers ribonucleic acids and P_1 and P_2 their final products. RG is the *regulatory gene* whose product R — the *repressor-in* the original form or modified by the *effector* F in a R' form, is able to react with the *operator* O. The transcription process may be initiated in the operator only if it is free from the repressor.

the invertebrate host, there is a transformation to the trypanosomal stage, the metacyclic form, infective for the vertebrate. When the blood trypanosomes are ingested by the invertebrate host, a second transformation takes place in the opposite direction. In the *lewisi* group of mammalian trypanosomes there is a second cycle of metamorphosis related to the multiplication activity in the vertebrate host (Fig. 2).

The cyclic process of trypanosome formation and reversion to the crithidial or/and leishmanial stage involves a complex rearrangement that is very incompletely known. From a morphological point of view the most remarkable aspects are the transformation and migration of the kinetoplast, the formation or disapearance of the undulating membrane and free flagellum and the variation in the over all aspect and mobility of the parasite. Some biochemical differences between the stages are known, for instance, for the cytochrome system in the *brucei* group (von BRAND [2]); antigenic differences between the crithidial and trypanosome stages were demonstrated by d'ALESANDRO [1] for *T. lewisi*, the former stage being insensitive to concentrations of ablastine that completely inhibited the multiplication of trypanosomes in the culture.

With respect to physiological properties and potentialities there is a marked difference between the metacyclic trypanosomes and the precedent crithidial/leishmanial forms. Thus for *T. cruzi*, only metacyclic trypanosomes survive after treatment with normal guinea pig serum at 37°C while crithidia are destroyed (MUNIZ & BORRIELO [11]). It seems excluded that crithidiae can develop in the vertebrate host, and tissue culture observations show that even at low temperatures crithidia cannot initiate the intracellular cycle. Furthermore it must be noted that only crithidiae divide in the invertebrate host, and that in the *lewisi* group, the blood trypanosomes also do not divide but only the leishmanial or crithidial forms.

In any case it is clear that the change from one form to another is accompanied by the expression of particular genetic potentialities. It is tempting to ask if the expression of the particular set of genetic information in one or another direction is not controlled by a basic regulatory mechanism similar to that proposed by JACOB & MONOD. We will discuss some data that could be interpreted in this way.

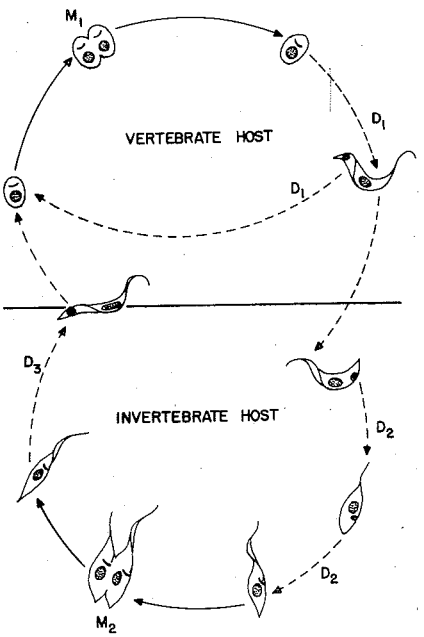

Fig. 2 — Life cycle of *Trypanosoma cruzi* — In continuous lines the multiplication process: M_1 in the vertebrate host under the leishmania form; M_2 in the invertebrate host under the crithidial form. Interrupted lines show the differentiation processes: D_1 — from leishmania to trypanosome and vice-versa in the vertebrate host; D_2 — from trypanosome to crithidia and D_3 — from crithidia to trypanosome in the invertebrate host.

Inducibility of cyclical changes

In many mammals trypanosomes, temperature is known to affect cyclic differentiation. Classic observations of CHAGAS [4], and others (see SILVA for references) showed that the shift from 37°C to room temperature in blood agar cultures of *T. cruzi* induces the transition of blood trypanosomes to crithidia. This transition takes 24 to 48 hours and can be easily followed in the blood extracted from infected animals and maintained "in vitro" at temperatures between 20° and 28°C, but is strongly inhibited at 5°C (SILVA [15]). In recent papers the inducing effect of temperature could be more properly analyzed because the authors employed culture media where the particular species of trypanosome studied was able to multiply in both forms — the blood trypanosome form or crithidial form typical of the invertebrate host. Thus, after inoculation of this culture medium with blood-agar cultural forms of *T. conorhini*, the development of blood trypanosomes was observed (DEANE & DEANE [5], DEANE & KIRCHNER [6]). This transition takes place only if the culture is maintained at 37°C when most crithidiae degenerate. If the same culture is maintained at 25°C — 28°C the crithidiae may divide normally, and metacyclic trypanosomes appear but not blood forms. With *T. lewisi* the maintainance of the culture at 37°C allows the multiplication of blood trypanosomes for a few days, but a shift to 28°C — 30°C induces the change to crithidiae that also divide (d'ALESSANDRO [1]). An interesting effect of high temperature in intracellular forms of *T. cruzi* was shown by NEVA et al. [14] in tissue culture. These authors, working with a Brazilian strain of *T. cruzi*, verified: (1) between 33° and 37°C the parasite could multiply intensively inside the cells and produce trypanosomes of the blood type; (2) at 38°C the multiplication could follow normally but the transition leishmania ⟶ trypanosome was blocked, the result being the accumulation of infected cells filled with leishmania bodies and the decrease of extracellular parasites. With another human strain ("Y" strain) we did not verify this effect but if the leishmania ⟶ trypanosome transition involves some thermosensitive critical event as defined by LWOFF & LWOFF [8] for the polio virus, we must expect strains with different thermosensitivities. It is necessary to prove that the thermosensitivity in the leishmania ⟶ trypanosome results from a mutational event in the parasite, but the relatively low temperature in which the effect was observed and the insensitivity of another strain points in this direction. It should also be important to look for an equivalent case in the invertebrate phase of the life cycle. namely thermosensitivity in the production of metacyclic trypanosomes.

The morphology of intra and extracellular parasites, especially the relative proportion of slender and broad trypanosomes, in tissue culture infected with a human strain of *T. cruzi* was dependent on temperature (TREJOS et al. [19]).

The possible action of chemical inducers is much less clear. There is very little information about nutritional requirements and metabolic activity of different stages of trypanosomes. The "morphogenetic factor" for *T. cruzi* demonstrated by MUNIZ & FREITAS [12] in red blood cells, may be a substrate necessary for the metabolism of the parasite, or a inducing element or both. Recent observations of DEANE & KIRCHNER (personal comunication) favor the existence of an inducing factor in red blood cells for the *T. conorhini* trypanosome ⟶ crithidia transition. The culture medium employed by these authors allows the multiplication of both stages of *T. conorhini* depending on the temperature and also allows the metacyclic ⟶ blood trypanosome transition when the temperature is shifted from 28 to 37°C. However, the opposite transition from blood trypanosome to crithidia, needs the presence of red blood cells, which could not be replaced by hemin or hemoglobin (DEANE & KIRCHNER [6]).

Specific metabolites may play a role in the transition processes for each phase of the cyclic evolution. We know that for *T. cruzi* the transition blood trypanosome ⟶ leishmania is induced by the intracellular medium after penetration of the parasite and that at the end of the multiplication period the reverse transformation is induced by the modified intracellular medium. According to MUNIZ & FREITAS [4] the transformation of leishmania to trypanosoma may be induced outside the cells in a medium composed of

guinea pig ascitic fluid and glucose but only in the presence of exudate cells. It seems likely that in each some cellular constituent or catabolite has an inductive effect.

In the invertebrate, metacyclic trypanosomes are produced only in some particular part of the digestive tract or its annexes: the hind gut for instance for the *lewisi* group and the hypopharynx for the *brucei* group.

The differentiation of crithidiae to trypanosomes in cultures of *T. cruzi* only occurs at the end of the exponential phase of growth. If, during exponential growth, the flagellates are transfered to incomplete medium the differentiation process takes place sooner. This suggests that starvation favors some physiological condition in which differentiation may occur (CAMARGO [3]).

The most interesting result in inducing form transition by chemicals was obtained by STEINERT [16,17] with urea. Low concentrations of urea administered to cultures of *T. mega* induced the transition from crithidiae to trypanosomes. The percentage of "inducible" crithidiae varied from 0 to 10% according to the age of cultures being greatest at the stationary phase of growth. The transformed cells, trypanosomes of the blood type, do not synthetize more DNA, while crithidiae present in the medium actively incorporated labelled thymidine (STEINERT & STEINERT [18]).

To summarize we may say that: 1) In each phase of the life cycle of trypanosomes, the parasites present some particularities that probably require the synthesis of particular proteins; 2) The transition processes are cyclic events, they involve some critical thermosensitive step(s), they can be induced by modification of the nutrient medium, and at least in one case can be induced by a simple substance that is not metabolized by the parasite. These facts point to a regulatory mechanism that controls the activity of specific genes, whose expression determines the differentiation processs.

If regulator gene(s) of this kind governing the differentiation process of trypanosomes does exist, we must expect to find "regulatory mutants" that have lost the capacity of differentiation in one or both directions, or in which this capacity is more narrowly limited in relation to temperature, pH etc. or less affected by specific inducers.

At this point we could speculate about the role of a similar mechanism for interpretation of the phylogenetic origin of trypanosomes like *T. equinum* that does not develop in the insect-host and *T. equiperdum* that is completely emancipated from the insect vector and adapted to direct transmission.

This approach is very important to understand the determinism of the life cycle of trypanosomes, as well as the morphological, biochemical and immunological characteristics of the different phases of the organism. It would not be surprising if, among the strains of *T. cruzi* that have lost their virulence after maintainance in laboratory for long periods of time, we should already have found some of these "regulatory mutants".

We want to emphasize the possible practical applications of these studies on regulation. The postulated possibility of isolating "regulatory mutants" that have lost their capacity to produce metacyclic trypanosomes or, alternatively, the understanding of conditions that control the metacyclic production would be of great practical interest for the development of vaccination processes in trypanosomiasis. We may also speculate that the understanding of the processes of differentiation in the vertebrate host could be of practical interest for instance in Chagas disease, if we could control the conditions inducing the intracellular leishmania trypanosome transition.

RESUMO

Diferenciação durante o ciclo evolutivo dos tripanosomas

Os processos de diferenciação no ciclo evolutivo dos tripanosomas são analisados à luz dos mecanismos de regulação da expressão de gens recentemente propostos para bactéria e vírus.

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