

## THE FINE STRUCTURE OF MEIOTIC CHROMOSOME PAIRING IN THE TRIPLOID, *LILIUM TIGRINUM*

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### INTRODUCTION

The elongate tripartite structure of synapsed homologous chromosomes at meiotic prophase was described by Moses (7) and by Fawcett (2) in 1956. A functional interpretation of this synaptonemal complex was put forward by Moses in 1958 (8) and by Coleman and Moses in 1964 (1). They proposed that the complex is "the morphological expression of the specific function of the pairing of homologous chromosomes that is a prerequisite of genetic crossing-over and cytological chiasma formation." In general terms, the basic significance of the complex has been confirmed by its regular occurrence in sexually re-

producing plants and animals. The involvement of the complex in genetic exchange was confirmed by Meyer (3, 4). Meyer reports that in two cases where genetic exchange does not take place (*Drosophila melanogaster* males and c3G females) no synaptonemal complexes occur. Similarly, no complexes were found in achiasmatic *Tipula ceasia* and *Phryne fenestralis* males. Chiasmatic *T. oleracea* males had normal complexes. Of direct relevance to this note is Meyer's observation (4) that in triploid *Drosophila* females normal synaptonemal complexes occur, but there are no indications that the third chromosome forms an individual single axial core. The unpaired leptotene chromosomes

of *Drosophila* females have no single axial cores either, and the complexes become apparent only after chromosome pairing (G. F. Meyer and R. C. King. Personal communications). In *Lilium longiflorum* and in *L. tigrinum*, unpaired chromosomes at meiotic prophase have single axial cores (5, 6). It has been shown in *L. longiflorum* that at zygotene the cores of a pair of homologous chromosomes approach each other to a distance of about 1000 Å and remain fixed in that parallel arrangement throughout pachytene (5). In this arrangement the cores are referred to as the lateral elements of the synaptonemal complex.

The axial cores and, at a later stage, the lateral elements of the complex reflect, in many respects, cytologically as well as genetically, the known behaviour of the meiotic prophase chromosomes. The fine structure of pairing relationships in a number of aberrant karyotypes (such as inversion heterozygotes, translocation heterozygotes, euploids, and polyploids) should reveal to what extent the core and the lateral element represent the chromosome in meiotic associations. This note reports that in the triploid lily, *Lilium tigrinum*, at meiotic prophase, the switch of pairing partners, well known from light microscopy, is reflected at the fine structural level by a switch of axial cores. That is to say, the core of homologue I is first found in a synaptonemal complex with the core of homologue II and then switches over to form a complex with the core of homologue III. In other cases, it was observed that the core of the third homologue can come in close contact, at least over short distances, with the synaptonemal complex of the other two homologues.

#### MATERIALS AND METHODS

Commercially available *Lilium tigrinum* ( $3N = 36$ ) bulbs were grown in field and greenhouse for a number of seasons. The relationship between bud length and meiotic stage through light microscopy and electron microscopy was determined as reported recently in a similar study on *Lilium longiflorum* ( $2N = 24$ ) (5). The pollen mother cells shown here came from buds between 21 and 25 mm in length (late zygotene, pachytene). The preparation of material for electron microscopy included 2 hr fixation in buffered 2% glutaraldehyde, postfixation in buffered 2% osmium tetroxide for 1 hr, dehydration through an alcohol series and propylene oxide, embedding in Epon, and staining with uranyl acetate and lead hydroxide. The material was sectioned with a diamond knife. Serial sections were deposited on oval-hole grids with Formvar, and they were then

carbon coated. The sections were recorded with a Philips EM 200 at 60 kv on Kodak electron image plates, usually at a magnification no higher than 16,000 at the plate. Sequences of 30–50 consecutive sections were used for this study.

The terminology introduced by Moses (8) is used here. The tripartite structure of the meiotic prophase bivalent is referred to as the synaptonemal complex. The two densely staining outer ribbons of the complex are the lateral elements, and the less dense ribbon between the lateral elements is the central element. The medial structures of the unpaired chromosomes at meiotic prophase are the axial cores.

#### OBSERVATIONS AND DISCUSSION

The tiger lily, *L. tigrinum*, was thought to be an autotriploid (11) because of its high number of trivalents at diplotene (see Fig. 1 *c*). Later reports indicate that all varieties of *L. tigrinum* are allotriploids (9, 10). A trivalent at diakinesis and metaphase I is the product of at least one partner switch and one cross-over in the paired segments on each side of the switch. The high frequency of trivalents at metaphase I (an average of about 10 out of a possible 12 per cell) in *L. tigrinum* suggests that partner switches and cross-overs commonly occur in this species.

At pachytene, sets of three homologous chromosomes are closely associated to form trivalents (Figs. 1 *a* and *b*). Generally one of the three homologues follows fairly closely the bivalent formed by the other two strands (Figs. 1 *a* and *b*, *BV* and *UV*). At a partner switch (Fig. 1 *b*, *PC*; Fig. 2), one of the homologues of a bivalent (*BV*) switches over to join the single univalent (*UV*), forming a new bivalent. It is usually assumed that even the sections marked *TV* in Figs. 1 *a* and *b* contain a bivalent and a univalent. The assumption is based on the observation that at a later stage (diplotene-diakinesis) homologues are associated in twos only. Electron micrographs indicate, however, that the association between the three homologues at pachytene can be as intimate as that between any two homologues (Figs. 3, 4). This intimate contact is here referred to as "partner fusion."

The axial core of the unpaired homologue has a typical morphology at pachytene and seems to differ from the core of the unpaired leptotene chromosome or the lateral element of the synaptonemal complex. A dense linear structure, about 150 Å wide, is median to a less dense zone, about 600–800 Å wide (Fig. 3 *d*). The entire structure is

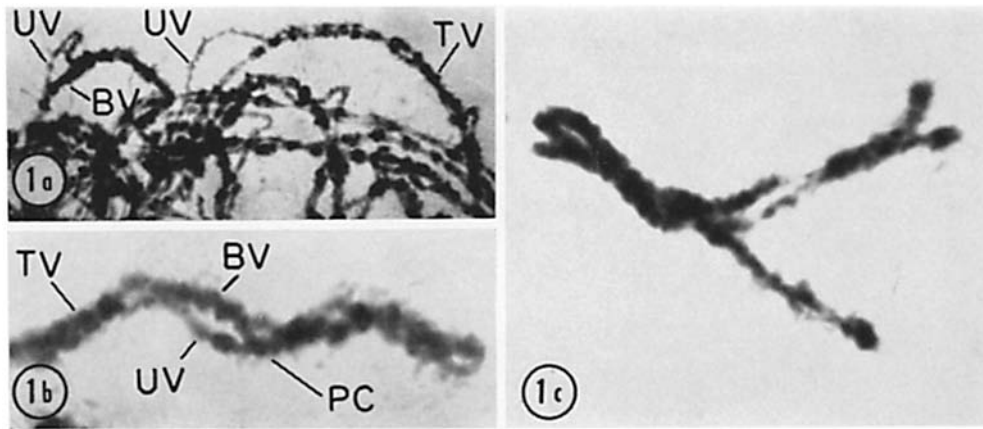


FIGURE 1 Light micrographs of triploid *L. tigrinum* pollen mother cells, stained with carmine and squashed. 1 a; The trivalent groups (TV) of three homologues consist of a bivalent (BV) and a univalent (UV).  $\times 1,100$ . 1 b; One homologue of the bivalent (BV) switches over and becomes paired to the univalent (UV) at the partner switch (PC).  $\times 2,000$ . 1 c; A trivalent at diplotene.  $\times 2000$ .

unevenly surrounded by densely stained chromatin. In single sections, cores up to  $7 \mu$  in length were found, and in serial sections the cores could be followed for longer distances. Details of these cores as well as a number of structural abnormalities which occur in the synaptonemal complexes of this triploid are described elsewhere (6). The asymmetry of the lateral elements seen in Fig. 4 and the unusually broad lateral elements shown in Fig. 2 are discussed in the same paper (6).

The chromosomes of about 1000 thin sections of pollen mother cells were searched for chromosome pairing. A total of four partner switches (Fig. 2) and nine partner fusions (Figs. 3, 4) were found. The drawing of the partner switch in Fig. 2 a is based on the serial sections shown in Figs. 2 b-e. Each segment in Fig. 2 a is labeled according to its presence in one of the serial sections. The axial cores and lateral elements are marked in solid lines; the central elements are dotted. The last part of synaptonemal complex number 1 (SC 1) lies entirely in the section in Fig. 2 c, where the divergence of the lateral elements can also be observed. Synaptonemal complex number 2 (SC 2) is composed of a lateral element in the section in Fig. 2 b, a central element in the section in Fig. 2 c, and the other lateral element in the section in Fig. 2 d. The homologues which participate in the partner switch are identified by roman numerals I, II, and III. It is clear that homologue II, represented by its axial core, is first the left member of synaptonemal complex number 1 with

homologue III, and then it switches over to become the right member of complex number 2 with homologue I. The structure of the cores suggests that, at an earlier stage, complex number 2 extended at least to where complex number 1 ends. That is to say, the core of homologue I, in the section in Fig. 2 d, has the features of a lateral element and only takes on a conventional univalent structure in the sections in Figs. 2 d and e. Similarly, the switchover area of homologue II has the characteristics of a lateral element.

While the partner switch shown in Fig. 2 confirms light microscope observations, the "fusion" shown in Fig. 3 could not be anticipated. In the section in Fig. 3 d, homologue I is a typical univalent. Homologues II and III are paired to form a synaptonemal complex which makes a  $180^\circ$  twist through the sections shown here. In Fig. 3 d the core of the univalent I touches upon the clear space of the synaptonemal complex. Serial sections, shown in Figs. 3 b-d indicate that the core, in fact, fuses with the core of homologue III.

The possibility of a univalent joining with a lateral element is also suggested in Fig. 4. The synaptonemal complex passes obliquely through the plane of section, and the univalent lies in the plane of the sections in Figs. 4 b and c. In the section in Fig. 4 c, the single core can be seen to approach the right lateral element of the complex. The section above and below Fig. 4 c showed a continuation of the complex as indicated in Figs. 4 b and d. No continuation of the univalent or its

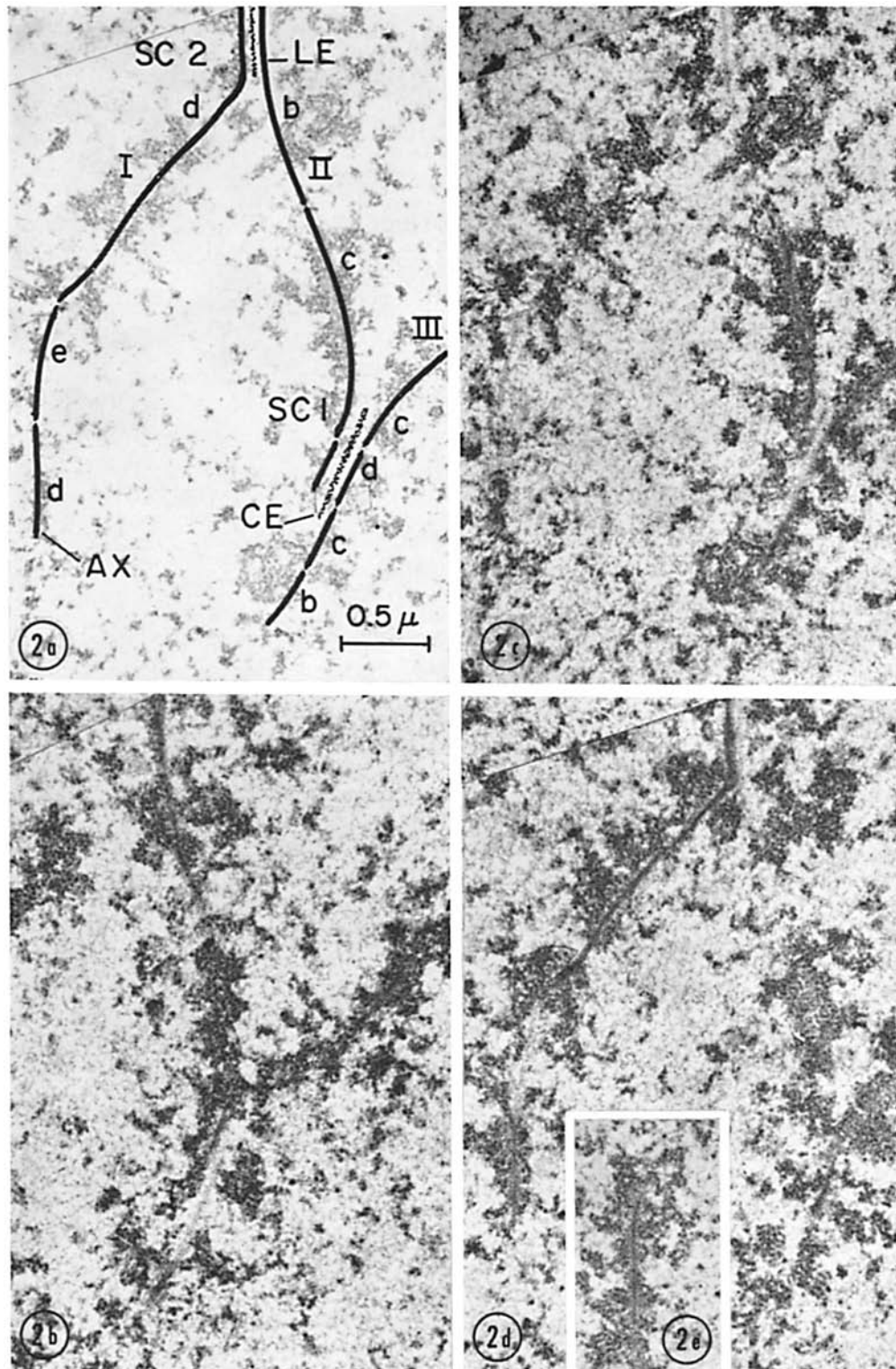


FIGURE 2 The fine structural detail of a switch of homologous partners in the triploid at pachytene. 2 a; The information from four consecutive sections is combined in this drawing. The axial cores (AX) and lateral elements (LE) are solid lines, and the lower case letters indicate from which figure each segment is copied. The central elements (CE) are dotted. The three homologues are marked by roman numerals (I, II, and III); they participate in two synaptonemal complexes (SC 1, SC 2).  $\times 25,000$ . 2 b-e are electron micrographs of consecutive serial sections. The edge of the section as well as a few stain crystals has been masked with inserts.  $\times 25,000$ .

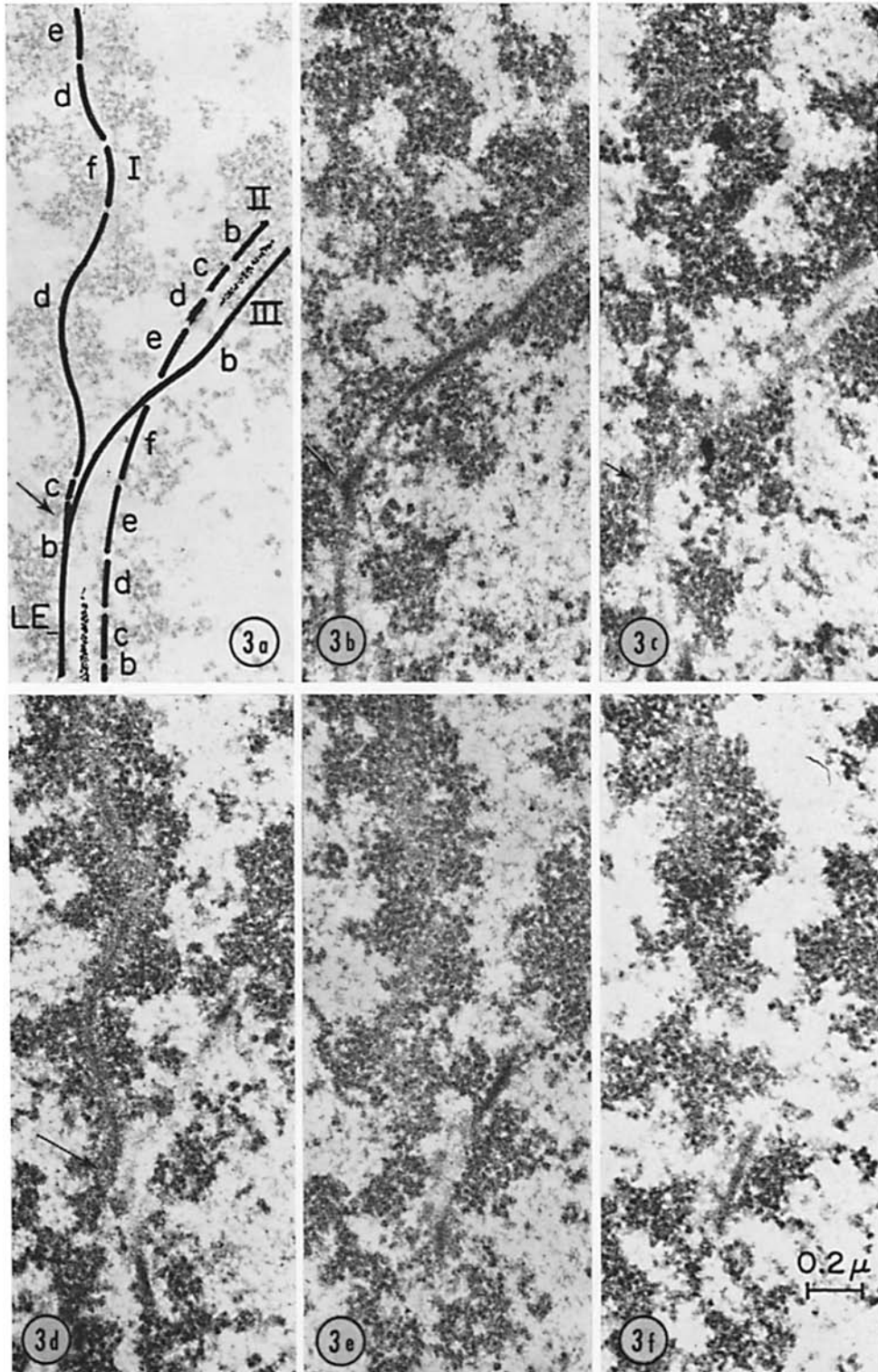


FIGURE 3 The fine structural detail of a partner fusion. 3 a; The interpretation of the drawing is as in Fig. 2 a. The cores of the homologues II and III form the lateral elements of a synaptinimal complex which bends and twists through the five consecutive sections. The core of homologue III is at one point (marked by arrow) in close contact with the core of the unpaired homologue I.  $\times 40,000$ . 3 b-f are electron micrographs of a partner fusion.  $\times 40,000$ .

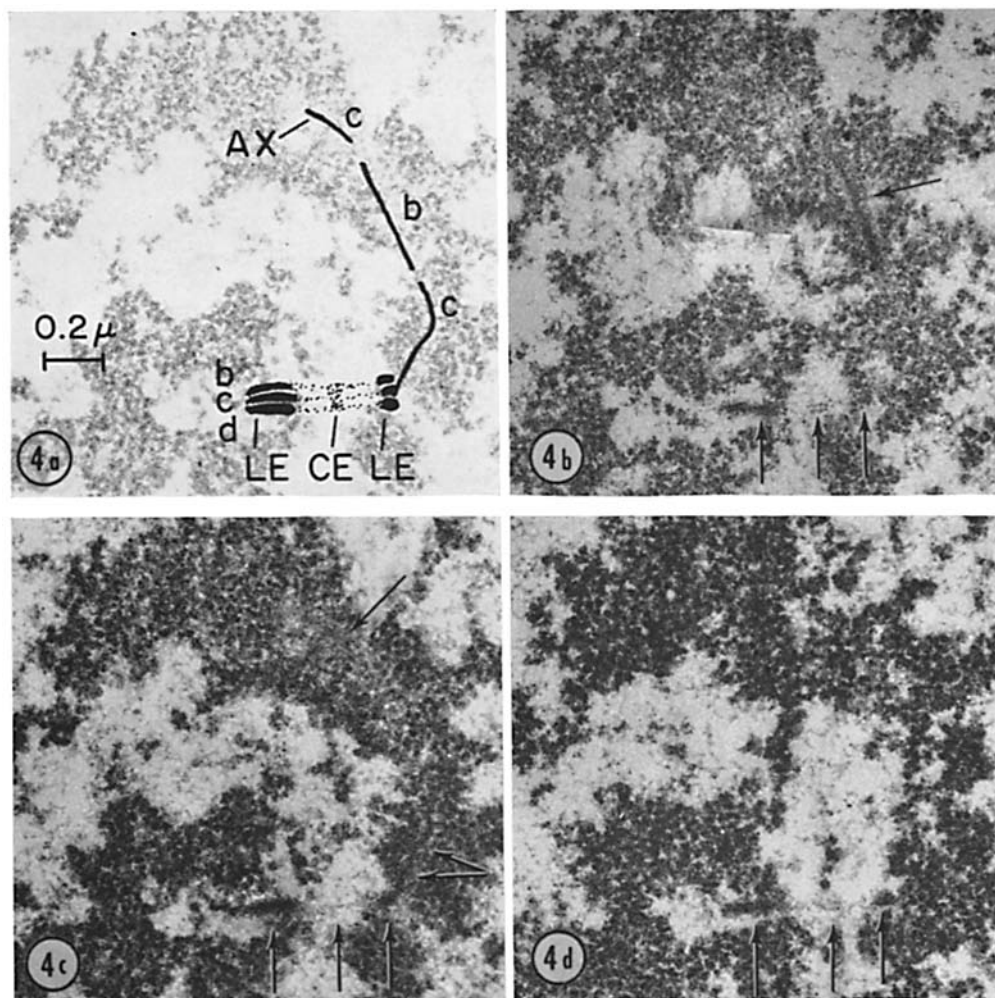


FIGURE 4 The fine structure of a partner fusion. 4 a; The synaptonemal complex is oblique to the plane of section so that the distance between the lateral elements (*LE*) is in excess of the normal 1000 Å. Serial sections indicate that this complex twists counter clockwise, but this is not shown in the drawing.  $\times 40,000$ . 4 b-d are electron micrographs of the partner fusion. *CE*, central element; *LE*, lateral elements; *AX*, axial core; arrows indicate these structures.  $\times 40,000$ .

core was detected up to  $1 \mu$  above and below the section in Fig. 4 c. The axial core of the univalent is difficult to distinguish if the core does not lie in the plane of section or nearly so. It is therefore possible that the core left the complex again in Figs. 3 and 4, but that this was not detected. From the evidence, it can only be said with certainty that close contact between the cores of three homologues can occur over short distances.

These observations of the pairing of homologues in the triploid lily confirm that, in the case of a

partner switch, as in the case of normal synapsis, the behaviour of the homologous chromosomes at meiotic prophase is mirrored at the fine structural level by the behaviour of the axial cores.

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