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On Principles of Organization of Polyreplicon Systems

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Summary. Principles for the acceptability of replicons and polyreplicon systems are formulated, based on the postulated single reproduction of all the components of these systems in each reduplication cycle. On this basis the diverse variants of properties inherent to these systems and their punctuation marks (replicators and terminators) are discussed. The problem of the existence of terminators of reduplication is also considered.

Variants of linear polyreplicon systems with identical punctuation are examined. Rules determining the acceptability of different aberrations are put forward. The following variants are unacceptable: inversions the ends of which lie in replicons with the same orientation; non-reciprocal translocations where the ends lie in replicons with opposite orientation; reciprocal translocations of two fragments differing in the orientation of the end replicons; all deletions. It is shown that polyreplicon systems in which all the replicons have the same orientation are subject to maximal losses due to aberrations, whereas systems consisting of two replicon groups equal in total length and having opposite orientation bear minimal losses.

The following conclusions are drawn from the comparison of these rules and the analysis of the experimental data: 1)-If terminators exist, replicons of eukaryote chromosomes, in which acceptable aberrations are found, do not overlap and have the same punctuation. 2)- In this case, the alternating orientation of replicons in mammalian chromosomes corresponds to minimal aberrational losses. A hypothesis is proposed for the evolutionary pathway along which linear polyreplicon systems of eukaryotes may have appeared. According to this hypothesis, circular replicons of prokaryotes have united by means of recombination into systems with subsequent selection of variants based on minimal aberrational losses.

In terms of modern replicon theory each unit of reduplication is initiated at a specific point on the chromosome (replicator), proceeds in a definite direction (orientation) and stops at another fixed point on it (terminator) (Jacob et al., 1963; Cairns, 1963; Sueoka and Yoshikawa, 1963; Pato and Glaser, 1968; Mosig, 1970; Ratner, 1972). It has been shown that in microorganisms the replicons of chromosomes and episomes are capable of uniting into polyreplicon systems containing a number of replicators and terminators. Such cases include the chromosomes of Hfr and lysogenic bacterial strains. The polyreplicon structure of eukaryote chromosomes has been demonstrated by autoradiographic studies (Huberman and Riggs, 1968). It is clear that certain properties of polyreplicon systems largely depend upon the mutual position and orientation of the replicons and upon the specific features of the replicators and terminators.

This paper considers some of the restrictions limiting variants of the acceptable polyreplicon systems, and the chromosome aberrations resulting from mutual orientation of the replicons and from the specificity of punctuation marks (replicators and terminators).

General Requirements for Polyreplicon Systems and Replicons. Features of Punctuation Marks

According to current genetic concepts, the major requirement to be fulfilled by a replicating system is its periodical exact reduplication. The failure of certain regions of the system to replicate results in loss of genetic material and, possibly, in death, whereas excessive replication of certain regions leads to the accumulation of redundant genetic material and frequently to gene imbalance, which is also undesirable.

Let us distinguish a class of replicating systems free from the drawbacks mentioned above. *Replicons and polyreplicon systems can be considered acceptable in terms of the reduplication concept, if, in each reduplication cycle, they reproduce each of their elements (including replicators and terminators) once and the system as a whole.* Based on these requirements, we can discard all the variants of replicons and polyreplicon systems which *systematically* lose or acquire any genetic elements through each reduplication cycle.

Cases with major and minor defects may be distinguished within the so-called "defective variants". Variants in which redundant punctuation marks arise and are lost during subsequent cycles of reduplication without impairment of function are termed *weakly defective.* Such variants are almost indistinguishable from the acceptable ones and we shall make no distinction between them. The other discarded variants are referred to as *strongly defective* or unacceptable.

In connection with the diversity of replicons and polyreplicon systems, we take into account the following characteristics:

1) properties of punctuation marks;
2) overlapping or non-overlapping of replicons;
3) circular or linear nature;
4) mutual orientation of replicons.

We can study the properties of punctuation marks, both actual and conceivable, bearing in mind their attributes and interrelationships:

a) the existence of pairs of punctuation marks inherent to one initiator;

b) symmetry or asymmetry of punctuation marks and the existence of bi-directional effects;

c) existence of terminators;

d) features of pairs of adjacent marks;

e) effects of mutual orientation and location of adjacent marks in pairs.

We shall treat only some properties of the linear polyreplicon systems.

Each replicon has a *specific punctuation* (replicator, terminator) and a *specific system realizing reduplication* (initiator), as postulated by Jacob et al. (1963) for replicons in microorganisms. This allows the independent replication of each replicon in each cell. Figuratively speaking, a replicon just "ignores" the punctuation marks of the other replicons. Polyreplicon systems organized into such replicons exhibit several variants of the reduplication process in which the functioning replication systems differ.

This point has been dealt with elsewhere (Jacob et al., 1963; Ratner, 1966; 1970; 1972).

It should be emphasized here that the initiators of replication (or multimere systems in which they are included) in each case seem to be specific for both punctuation marks. From this, we shall further assume that *in each replicon the initiator is specific for each pair of punctuation marks, replicator-terminator*. Hence, a segment of DNA chain, limited by a replicator and a terminator which do not form a specific pair and are not recognized by a single initiator, *does not represent a completed replicon.*

There is another acceptable variant distinguishing a replicon: a DNA segment bounded by *two replicators* having the same specificity and opposite orientation providing simultaneous (or almost simultaneous) initiation of two reduplication processes proceeding from opposite direction. In this case, the terminal signal is unnecessary if DNA polymerases are liberated after these processes have met (Huberman and Riggs, 1968).

It is assumed that the replicator and terminator are regions of double-stranded DNA free from defects, the size of which probably does not exceed the molecular size of the recognizing enzyme or multienzyme complex. The specificity of the punctuation marks is provided by the arrangement of monomeres, which is unique within replicons. Starting from the replicator, both directions of replications are "equivalent", as a chemist would understand it. Nevertheless, it has been observed that in microorganisms (for instance, in chromosomes and episomes of *E. coli*, *S. typhimurium*, *B. subtilis*, genome of T4 phage) replication is always unidirectional (Jacob et al., 1963; Cairns, 1963; Sueoka and Yoshikawa, 1963; Pato and Glaser, 1968; Mosig, 1970; Ratner, 1972). Hence, it is supposed that the choice of direction is pre-set by the orientation of the replicator, but the replicator can not exert a bidirectional effect. Because the function of the replicator (including the orientation of the initiated reduplication) is determined by the sequence of the monomere pairs composing it, the *replicator is asymmetrical relative to its inversion in double-stranded DNA*.

This conclusion may be correct, but it is not the only possible one. In fact, the data on microorganisms concern cases lacking proof that replicators function by themselves; other adjacent punctuation marks and free ends of DNA molecules may lie near them. It is just as plausible to consider each punctuation mark symmetrical so that the choice of the direction of replication is determined by the effect of adjacent pairs or the end replicator. *Bidirectional replication* of DNA regions, postulated by Huberman and Riggs (1968) in mammals and by Schnös and Inman (1970) in λ phage, does not solve the problem. These regions may be interpreted as replicon pairs with opposite orientation having either one common symmetrical replicator, or two adjacent replicators oriented in opposite directions.

However, it is easy to see that both suggestions lead to similar results. For this reason, we shall not distinguish between these two variants. Variants with pairs of asymmetrical marks may well be substituted for their symmetrical equivalents. Fig. 1 (a—c) shows variants of acceptable pairs of adjacent asymmetrical punctuation marks.

It should be noted that the existence of a terminator has so far been proved only in λ-phage (Ratner and Kulitchkov, 1971). From the studies on intracellular branched circular DNA molecules performed by Schnös and Inman (1970), it appears that the replication process in λ DNA originates at one point, but moves both to the left and right and never intersects a certain region which, thus, should comprise the bidirectional terminator. According to the data of Imae and Fukusawa (1970), the induction of λ N^- and Q^- mutants initiates reduplication in the bacterial chromosome itself, the host region being

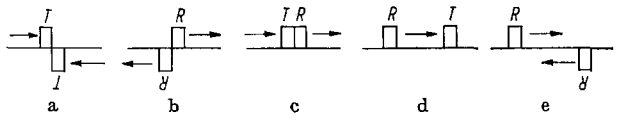

Fig. 1. Acceptable pairs of punctuation marks. Horizontal line — DNA strands, rectangles — punctuation marks. Above the line punctuation marks of right orientation, below — punctuation marks of left orientation (their indexes are overthrown). (a), (b), (c)-acceptable variants of adjacent marks, (d), (e)-pairs of punctuation marks of identical specificity limiting the replicons

copied only to the left beyond the prophage in the vicinity of the *gal* genes and not involving the *bio* genes at the right. Hence, there is no terminator to the left of the bidirectional replicator of λ prophage, whereas it exists to the right.

It is concluded that *λ phage has two replicons oriented in opposite directions and that the replicators and terminators limiting them are symmetrical, i.e. exert bidirectional effects.* In all the other cases there are no data favouring the existence of terminators. However, in referring to polyreplicon systems of eukaryotes we shall always assume that terminators exist.

Linear Polyreplicon Systems

The features of punctuation marks, which we have listed above, call for the consideration of variants of linear polyreplicon systems and the rearrangements which would be acceptable in alternative situations. Let us analyze linear polyreplicon systems *with identical punctuation marks.* It is assumed *that terminators exist.* Such systems imply that *adjacent punctuation marks of one replicon and overlapping replicons would be unacceptable.* Let us examine variants of chromosome aberrations acceptable in such systems. Inversions, translocations and duplications occurring with single replicons or involving unimpaired replicons with their punctuation marks (or their groups) are always acceptable in any polyreplicon system of the linear type.

We proceed now to aberrations affecting different replicons. In each case there are two variants of the mutual orientation of replicons in which aberrations occur (or the ends of rearrangements are located): similar or opposite. In inversions and deletions there is only one way of uniting the ends; in translocations and duplications there are several ways.

The results of the analysis are formulated in the following rules:

1) *Inversions,* the ends of which lie in replicons having the same orientation, are unacceptable (Fig. 2).

2) *Nonreciprocal translocations,* the ends of which lie in replicons of opposite orientation, are unacceptable.

3) *Reciprocal translocations* of two fragments are unacceptable when the ends of one fragment lie in replicons of the same orientation, and the ends of the other are located in replicons of the opposite orientation.

4) *Deletions* are always unacceptable if the formed fragments remain linear.

In other types of orientation there are variants of corresponding rearrangements acceptable from the point of view of reduplication. Among duplications and two-break reciprocal translocations, acceptable variants are always present.

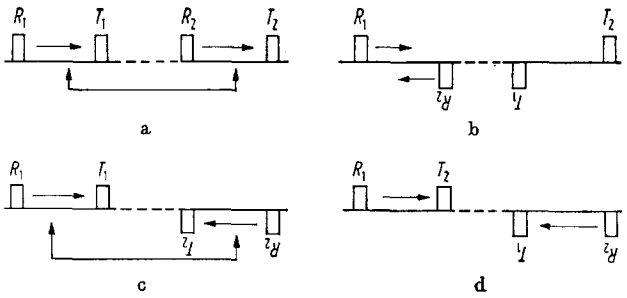

Fig. 2. Variants of inversions in polyreplicon systems: (a)- initial replicons with same orientation, (b)- unacceptable inversion, (c)- initial replicons with opposite orientation, (d)- acceptable inversion. Arrows indicate boundaries of inversions

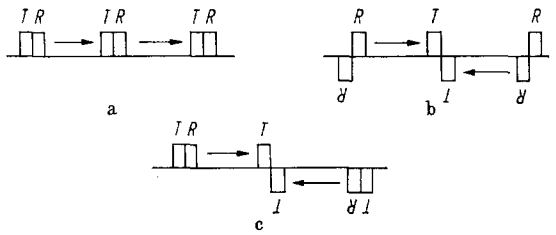

Fig. 3. Variants of linear polyreplicon systems: (a)- all replicons have the same orientation, (b)-adjacent replicons have opposite orientation (variant of Huberman and Riggs, 1968) (c)-opposite oriented replicons arranged into two blocks

From these elementary rules important conclusions may be drawn, some of which can be verified experimentally. Here are examples:

a) If acceptable aberrations exist in an acceptable polyreplicon system, then, at least in those replicons where united regions are located, *the punctuation marks are equally specific.*

b) Acceptable *inversions change the orientation of all the replicons which they comprise,* excluding the two extreme ones, where breaks and joints are located.

c) *Total losses* of polyreplicon systems due to the occurrence of unacceptable inversions and translocations *depend upon the ratio of the total lengths of replicons with left and right orientation.*

The last rule can be examined in detail. Let the replicons of a polyreplicon system have an identical orientation (Fig. 3 a): in this system inversions are unacceptable and the number of acceptable nonreciprocal translocations is maximal. If replicons of a polyreplicon system form two groups equal in total length but of opposite orientation (Fig. 3 b, c), then the number of acceptable inversions is maximal and the number of acceptable translocations is minimal.

The following designations were introduced: l — total length of a polyreplicon system; l_1 — total length of replicons with left orientation; $l - l_1$ — total length of replicons with right orientation; $d \ll 1/l$ — mean probability for the occurrence of breaks per unit of length. Then the total prob-

ability for the occurrence of unacceptable inversions and translocations is given by

$$\psi(l_1) = \alpha\, d^2\,[l_1^2 + (l - l_1)^2] + \beta\, d^3\, l\, l_1\, (l - l_1)\,,$$

where α and β are constants of the probability of double and triple unions of free ends with $\alpha \gg \beta\, l\, d$.

It is easy to see that this expression is minimal at $l_1 = l/2$. *Thus, losses in the polyreplicon system resulting from unacceptable inversions and translocations are minimal if the total lengths of the groups of "right" and "left" replicons are equal.*

Based on these considerations, some conclusions concerning eukaryote chromosomes follow.

Polyreplicon Systems of Eukaryote Chromosomes

All the replicons of the chromosomes should be adequate in the framework of the replication concepts in order that the linear polyreplicon chromosomes produce exact copies through the cell cycle; in other words, they should be limited by pairs of punctuation marks, "replicator-terminator", *of the same specificity and orientation.* Replicons fulfilling these requirements *do not overlap.*

The conclusions concern all the existing chromosomes in which it has been proved that the DNA content per chromosome (and desirably in all chromosome regions) *exactly doubles through each cell cycle.* This is particularly relevant to the numerous bands of the polytene chromosomes in Dipterae (Lindsley and Grell, 1968; Kiknadze, 1972). Another line of evidence comes from the autoradiographic studies on the mechanisms of DNA replication in the chromosomes of the Chinese hamster and HeLa cells, confirming the doubling of DNA content in many unrecognized replicons (Huberman and Riggs, 1968).

Determination of the orientation of replicons in chromosomes of eukaryotes presents a problem. A direct contribution to its solution has been provided by means of pulse autoradiography only in the chromosome DNA of fibroblasts in the Chinese hamster and human HeLa cells (Huberman and Riggs, 1968). This proved that *over 90% of adjacent reduplication processes are oriented in opposite directions and 3% are unidirectional or may be interpreted as so.* If terminators exist, this situation is similar to the one presented in Fig. 3(b), *which provides minimal losses due to aberrations. However, deviations from the extreme unified principle of the orientation of replicons are acceptable.*

If all the replicons contain terminators then the existence of acceptable aberrations serves as proof *of the identity of punctuation marks,* at least in the replicons in which the joined ends of aberrations are located.

The rules formulated above may be used in the analysis of the orientation of replicons. *Any two replicons in which the ends of a viable inversion are located have an opposite orientation. Any two replicons in which the ends of viable nonreciprocal translocation are located have a similar orientation.* With a wide range of viable aberrations it is possible to have a good idea of the topography of the orientation of replicons. Moreover, estimates of the relative number of "left" and "right" replicons obtained on this basis can be verified autoradiographically. Carrying the analogy further, the variant of replicon orientation of Huberman and Riggs (1968) can be extended to other organisms, such as Dipterae.

Unacceptable rearrangements present an interesting area for future research. Despite being lethal in the homozygous state, these rearrangements can be cultured and studied in the heterozygous state. It is important that if in an unacceptable inversion certain segments do not replicate, then this region is bounded by two terminators, and the pattern produces the impression of the deletion of two regions of a genome lying close to the terminators (Fig. 2b, right). The size of the deletion varies depending upon the location of the points of breakage within the two replicons, but its right end will always break off at the right terminator T_2 and its left end at terminator T_1. In other words, the boundaries set by definite loci limiting the deficiencies at the right and at the left may indicate the actual position of the terminators and the orientation of corresponding replicons.

The acceptability of rearrangements is also restricted by their position relative to the centromere. This is beyond the scope of this paper, which describes acceptability only in connection with reduplication.

Possible Evolutionary Pathway for the Rise of Linear Polyreplicon Systems

If terminators exist, *the striking features of chromosome organization, providing minimal aberrational losses, are the frequent alternation of adjacent replicons with opposite orientation and the existence of bidirectional sites of initiation and termination (which have been established in the Chinese hamster and man).* A criterion is therefore conceivable, which favours systems of this type (Fig. 3 b) compared with systems subject to heavier losses (Fig. 3 a). It is unclear, so far, whether this is a general principle underlying chromosome organization, or whether in nature there exist other systems equally successful in minimizing losses of the types shown in Fig. 3(c), or intermediate systems.

Only extensive autoradiographic studies on the replication of chromosome DNA in eukaryotes may provide a decisive answer, but a survey of possible evolutionary pathways along which certain variants arose is, perhaps, an approach to the problem.

In the first place, symmetrical punctuation marks could not have appeared later than the primary polyreplicon systems. Thus, either primary prokaryotes, the precursors of the present eukaryotes, had symmetrical punctuation marks, or they arose directly

in the course of formation of the primary eukaryotes. The existence of bidirectional reduplication in λ phage substantiates the former suggestion (Schnös and Inman, 1970; Stevens et al., 1971).

However, we shall analyze the most unfavourable case. From suggestions for the features of this process, it appears that there is a simple pathway for the formation of adjacent pairs of similar punctuation marks, resulting in the Huberman-Riggs variant.

Suppose that the primary prokaryotes, *which had separate circular replicons* just as their present descendants have, were ancestors of the primary eukaryotes. To obtain the system of Huberman-Riggs, it could be that the cells of prokaryotes *had several (at least two) replicons with identical punctuation marks.*

If this were so, then adjacent punctuation marks of different replicons would be *mutually homologous regions* and could form a synapse with subsequent recombinational exchange according to Campbell's model (Campbell, 1962) (Fig. 4a, b). Fig. 4(b) shows that in this case the usual exchange leads to the assembly of replicons into one circular two-replicon system *with the same orientation of replicons.*

However, there is another way of joining the broken ends, depicted in Fig. 4(c). Let us assume that the breaks in both DNA strands are homologous and are situated exactly between the replicators and terminators (Fig. 4d). Such an event is certainly very unlikely to occur. However, the existing procaryotes are known to have specific systems of recombination, determining with high probability the exact location of the break. An example is the system of integration in λ phage (Dove, 1968). Let us further assume that *the left ends and right ends of breaks join separately, i.e., similar terminators and replicators with opposite orientation* prove to be adjacent in the polyreplicon system (Fig. 4c, d). The system formed then *consists of replicons with opposite orientation.* There are no other variants of crossed reunion.

Systems of type (b), Fig. 4, *have maximal losses* due to unacceptable aberrations, whereas *systems of type* (c) *bear minimal losses.* It is evident that natural selection favours type (c) in populations with such genetic diversity. If conditions are equal in all other respects, this eventually eliminates type (b) from the population.

The nascent and selected circular double-replicon systems conform to an interesting pattern because during their subsequent union *they do not lose their acquired features, but retain them irreversibly* and increase the number of replicons by means of recombination. The gene content of uniting replicons or polyreplicon systems may be similar or different. Evidently, the doubled regions may diverge.

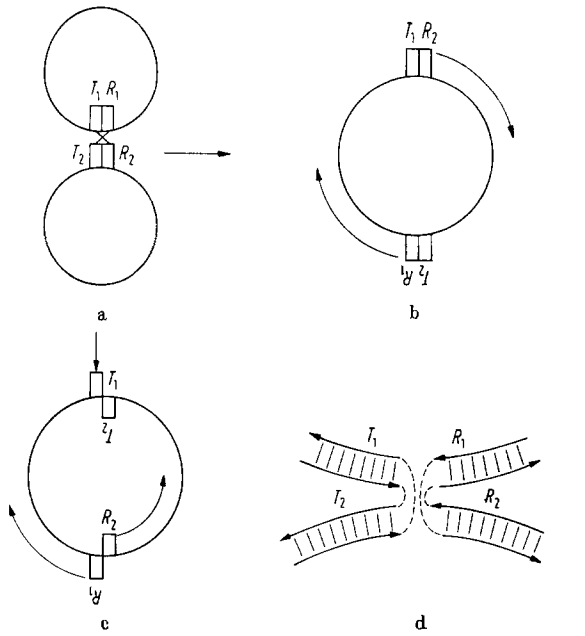

Fig. 4. Possible evolutionary pathway for the rise of alternating orientation of replicons in eukaryotes from circular replicons of prokaryotes. (a)-synapse of circular replicons with similar punctuation, (b)- circular two-replicon system with similar orientation of replicons obtained by means of usual recombination, (c)-circular two-replicon system with opposite orientation of replicons obtained as a consequence of unusual joining of ends, (d)-diagram of unusual joining of ends of breaks

Finally, to obtain a linear polyreplicon system it is necessary to break the circle between two adjacent replicators or terminators. The outcome is the variant of Huberman-Riggs.

Thus, the system of Huberman-Riggs is developed from circular replicons in a simple way, and selection for minimal losses due to aberrations at the initial stage of union is sufficient for its fixation. Subsequently the systems retain their structure automatically and perpetuate essential features. Therefore, it would be anticipated that the polyreplicon systems of Huberman-Riggs are of fundamental importance and widely distributed in nature.

The foregoing discussion does not exclude other possibilities. For instance, polyreplicon systems with identical replicon orientation (see Fig. 4b), uniting as shown in Fig. 4(c), may produce other variants of polyreplicon systems having minimal aberrational losses (see Fig. 3c). The problem is to survey the merits and disadvantages of all the variants arising and to discern the stable consequences of evolution.

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Note added in proof

The suggested way for the arisal of symmetrical punctuation marks by means of the recombinations is not unique. The other possible way is the same joining of the copies of replicons after replication was over. E. A. Abeleva had obtained this result independently of us (Ontogenes (russ.) 2, 235–245, 1971).

M. Masters and P. Broda (Nature, New Biology 232, 137–140, 1971) found some facts to prove the bidirectional pattern of replication in *E. coli* chromosome. It may be shown that most of experimental data (Cairns, 1963; Pato and Glaser, 1968; et al.) are in good correspondence with this hypothesis.

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