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Hierarchic Structure of Reproductive Methods

Marina Dan-Sohkawa

Department of Biology, Faculty of Science,
Osaka City University, Osaka 558, Japan.

生殖法の階層構造

大阪市立大学理学部　団　まりな

ABSTRACT

Hierarchic relationship is revealed between four reproductive methods, amitosis, mitosis, sexual reproduction and ontogenesis. Functional structures of each of the reproductive methods are analyzed and used for comparison. Relative complexity and hierarchic relationship between these methods are evaluated by processing them through two criteria, the "relation of inclusion" and the "acquisition of new function(s)".

1.INTRODUCTION

Hierarchic relationships of three cellular entities, prokaryotic, haploid and diploid (Dan-Sohkawa, 1993), and of different animal body structures (Dan-Sohkawa, 1992) have been revealed recently. It is the purpose of this communication to show that such relationship is also found in a functional aspect of living organisms, i.e., in reproductive methods.

2.THE WAY TO DEFINE RELATIVE COMPLEXITY

For analyzing the presence of hierarchic relationship between living entities, it is crucial to correctly judge the relative complexity of the entities in concern. The way to

define relative complexities of living entities have been presented elsewhere (Dan-Sohkawa, 1992, 1993). In brief, two living entities are processed through two criteria. In the first criterion, called the "relation of inclusion", it is checked whether one of them is made of the other, or, to put it inversely, whether one is included in the other. If we find a relation in which an entity A is containing, or is made of the other entity B, it is judged that A is more complex than B. And if A is made directly of B, we can say that A is one level more complex than B.

In the second criterion, called the "acquisition of new function(s)", the entity which was judged to be more complex is further checked for possession of function(s) newly acquired by it, and, therefore, not found in the lower, constituent entity. It is only after these two criteria being fulfilled that an entity will be concluded to be truly more complex than its competitor.

In order to compare between different methods of reproduction, i.e., amitosis, mitosis, sexual reproduction and embryonic development or ontogenesis, it is necessary to make clear which features are to be used for comparison. I shall start my argument by analyzing functional elements of each of these reproductive methods and define their structures. It will be through these defined features that they will be evaluated in terms of the two criteria.

3. STRUCTURE OF AMITOSIS

The simplest method of reproduction found among living organisms is amitosis. This is the method of reproduction belonging to prokaryotic entities, including the organelles of eukaryotic cells, mitochondria and chloroplasts.

Amitosis begins with duplication of the "attachment" protein, a membrane protein binding the DNA molecule to the cell membrane (Fig. 1 a) at its *oriC* locus, the site of origin of replication (Hendrickson *et al.*, 1982). DNA replication starts

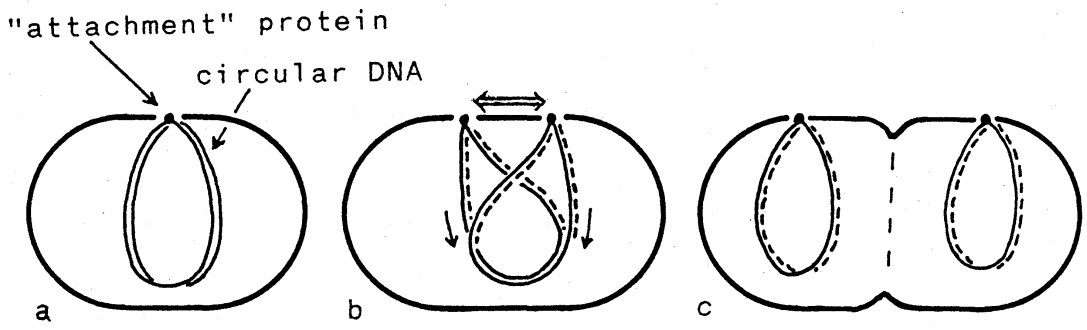

Figure 1 The process of amitosis

from this point and proceeds along the DNA molecule in two opposite directions (Fig. 1 b: arrows). On each replication fork, a dimer of DNA polymerase III holoenzyme is considered to form a complex with DNA primase, DNA helicase and a number of helix unstabilizing protein molecules (Alberts *et al.*, 1989). Meanwhile, the two "attachment" protein molecules are separated from each other by addition of new cell membrane between them (Fig. 1 b: double arrow). Finally, a new partition is built between the duplicated DNA molecules (Fig. 1 c).

This process of amitosis is interpreted to be composed of two mutually indifferent mechanisms, namely, "registration of DNA molecule to cellular membrane" and "the DNA replication complex". Accordingly, amitosis is defined here as

$$\text{AMITOSIS} = (\text{registration of DNA to cellular membrane}) + (\text{DNA replication complex}) \quad \ldots\ldots\ldots\ldots \quad (1)$$

4. STRUCTURE OF MITOSIS

Mitosis is the method of reproduction adopted by eukaryotic entities, including both haploid and diploid cell.

The major enzyme working in replication of DNA molecules in eukaryotic cell is DNA polymerase α. This enzyme is considered to be the functional homologue of DNA polymerase III of the prokaryotic cell by the following two reasons. Firstly, it is composed of several different polypeptides, all of which are re-

quired for its function. In other words, it is only functional in the form of a holoenzyme. Secondly, it functions in cooperation with the same proteins mentioned above, i.e., in the form of the "DNA replication complex" (Watson *et al.*, 1987).

The DNA molecules, on the other hand, are bound to the inner membrane of the nuclear envelope by three types of nuclear lamins during interphase (Gerace *et al.*, 1978; Hancock and Hughes, 1982; Lebkowski and Laemmli, 1982), and probably during S-phase, while they are being replicated (Fig. 2 a). As the cell enters into mitosis, M-phase, the chromosomes are released from the nuclear envelope (Gerace and Blobel, 1980) and begin to condense into metaphase bivalent chromosomes (Fig. 2 b). The nuclear envelope fragmentates eventually (Fig. 2 c) and the chromosomes split into halves and are carried away to two opposite poles by the tubulin system, known as the mitotic apparatus (Fig. 2 c, d). Subsequently to decondensation of the chromosomes and reassembling of the nuclear envelope, the DNA molecules are bound again to the nuclear envelope (Fig. 2 e, f). Mitosis is completed by cytokinesis as the result of action of the contractile ring, a loop of actin-myosin bundle formed around the equator (Fig. 2 e: shown in cross section).

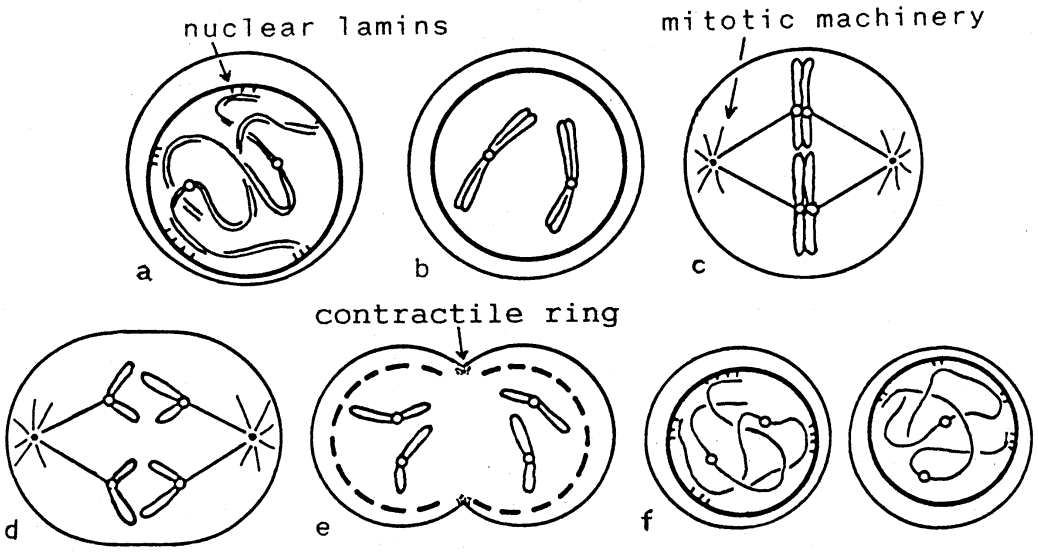

Figure 2 The process of mitosis

The events which take place during the M-phase, as just explained, can be divided into two major mechanisms. The first is "binding and releasing of DNA molecules to and from the nuclear envelope by nuclear lamins". The second is the "mitotic machinery" including both the mitotic apparatus and the contractile ring. The purpose and function of the two mechanisms are fundamentally independent from one another, although they are controlled to function in deep coordination during mitosis. The same is also true for the "DNA replication complex".

"Binding of DNA molecules to the nuclear envelope" is further interpreted as "registration of DNA molecule to cellular membrane". In contrast to the situation concerning DNA polymerase III and α, nothing is known about the relation between the "attachment protein" and the nuclear lamins. Only one of the three immunologically related nuclear lamins, B, is a membrane protein, while the other two, A and C, are cytoplasmic, intermediate filament proteins (Aebi *et al.*, 1986; McKeon *et al.*, 1986). Some authors, however, consider that the portion of the cell membrane associated with the prokaryotic DNA molecule, was drawn inside the cell at the time of evolution of the eukaryotic nucleus (Allsopp, 1969; Bell, 1970; Sato, 1988). In any event, I think it is safe to consider the nuclear lamin system as a functional extension of that of the "attachment protein" in controlling an increased amount of DNA molecules.

Accordingly, mitosis is described here as

MITOSIS = (registration of DNA to cellular membrane)
+ (DNA replication complex)
+ (mitotic machinery) ..... (2)

5. STRUCTURE OF SEXUAL REPRODUCTION

This is the second mode of reproduction belonging to eukaryotic cells, in which an organism switches its bodily status between haploidy and diploidy (Dan-Sohkawa, 1993).

The process of sexual reproduction is composed of two dis-

tinct parts, namely conjugation and meiosis. Therefore,

SEXUAL REPRODUCTION = (conjugation) + (meiosis) .... (3)

"Conjugation" is a relatively simple process in which two haploid cells fuse with one another, mingle their genetic material and become a diploid cell (Fig. 3).

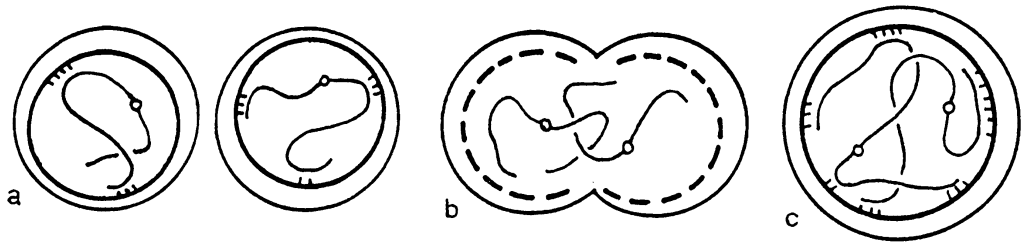

Figure 3 The process of conjugation

The process of "meiosis", on the other hand, is much more complex. It can be divided into four mutually independent mechanisms. They are (1) premeiotic DNA replication, under (2) the control of nuclear lamins, (3) chromosome pairing, or formation of synaptonemal complex between homologous chromosomes, and (4) mitotic machinery.

"Premeiotic DNA replication" is somewhat different from regular mitotic DNA replication. It leaves some 0.2 to 0.3 % of the total DNA unreplicated. The unreplicated sites are distributed throughout the lengths of the chromosomes in small pieces of about 50 kbp (zyg-DNA) and 160 kbp (P-DNA)(Hotta and Stern, 1981; von Wettstein *et al.*, 1984). This DNA replication, however, is interpreted here as a process equivalent to that of mitosis for the reason that the bulk DNA is replicated by DNA polymerase a in both meiosis and mitosis (Watson *et al.*, 1987). In contrast, the synthesis of at least P-DNA, which is synthesized later in pachetene, is catalyzed by the repair type polymerase, i.e. DNA polymerase β (Hotta and Stern, 1981; Hotta *et al.*, 1985)

Near the end of the premeiotic S-phase, there begins the synthesis of enzymes specific to meiosis, such as L-protein and m-rec protein. The former is an endonuclease which introduces a single nick to zyg-DNA (Hotta *et al.*, 1984; Stern, 1986) while the latter catalyzes duplex formation between the strands of homologous chromosomes (Hotta *et al.*, 1985). These enzymes, along with other meiosis specific enzymes, function variously in the synthesis of zyg-DNA and P-DNA, meiotic pairing of chromosomes, chiasma formation, and meiotic recombination. I will collectively call these processes the "synaptonemal events".

During chromosome pairing, DNA molecules are even more deeply committed with the cellular membrane than during mitosis. At leptotene, chromosomes start to attach randomly to the inner membrane of the nuclear envelope at their ends (= telomeres) using the lateral component (= one of the structural components of the synaptonemal complex)(Fig. 4 a). Subsequently, short stretches of synaptonemal complex start to appear at independent sites along the chromosome pair (von Wettstein *et al.*, 1984). Furthermore, at least two of the meiosis-specific, DNA-binding proteins, L-protein and R-protein, are membrane proteins of the inner nuclear envelope (Hotta *et al.*, 1984; Stern, 1986).

The tetrad chromosomes, the result of synaptonemal complex formation (Fig. 4 b, c), are subsequently segregated into univalent chromosomes through two rounds of meiotic division (Fig. 4 d, Fig. 2 b-f except with only one bivalent chromosome). The machinery of this division, as it may be seen, is no different from that of mitotic division.

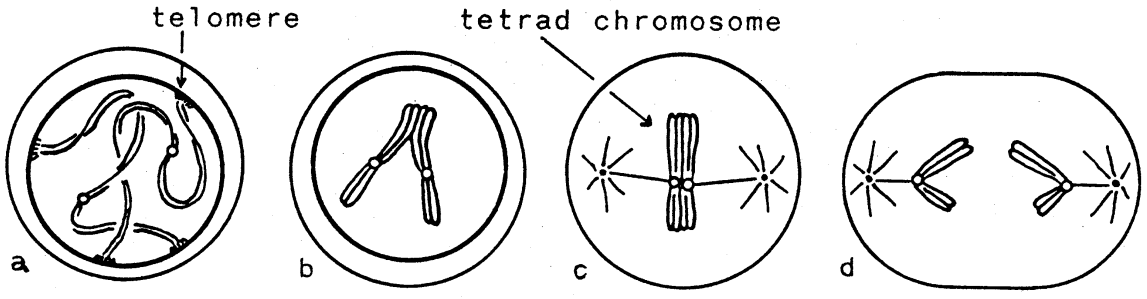

Figure 4 Behavior of chromosomes during synaptonemal events

Meiosis, therefore, is described here as

MEIOSIS = (premeiotic DNA replication)
+ (registration of DNA to cellular membrane)
+ (synaptonemal events)
+ (mitotic machinery) ..... (4)

6. STRUCTURE OF EMBRYONIC DEVELOPMENT OR ONTOGENESIS

Embryonic development is the method of reproduction of multicellular organisms. Its hierarchic structure has been revealed, recently (Dan-Sohkawa, 1992). In brief, it begins with the formation of gametes and their fertilization, i.e., with "sexual reproduction" (Fig. 5 a, b). The fertilized egg cleaves and develops into a blastula, a multicellular body with an "epithelial organization" (Fig. 5 c). Excretion of extracellular matrix into the space surrounded by the epithelium, the blastocoel, and migration of mesenchyme cells therein (Fig. 5 d) transform the embryo into a body one level more complex than blastula. Mesenchyme gastrula is characterized by its "mesenchymal coelom", or the primitive coelom, in which major organs are formed. Formation of "epithelial coelom", or deuterocoel (Fig. 5 e), raises the complexity of the structure of the embryonic body by another level.

Ontogenesis is defined, accordingly, as

ONTOGENESIS = SEXUAL REPRODUCTION
+ EPITHELIALIZATION
+ MESENCHYMAL COELOM FORMATION
+ EPITHELIAL COELOM FORMATION ..... (5)

Structure of all four reproductive methods defined, we are now ready to discuss their hierarchic relationships.

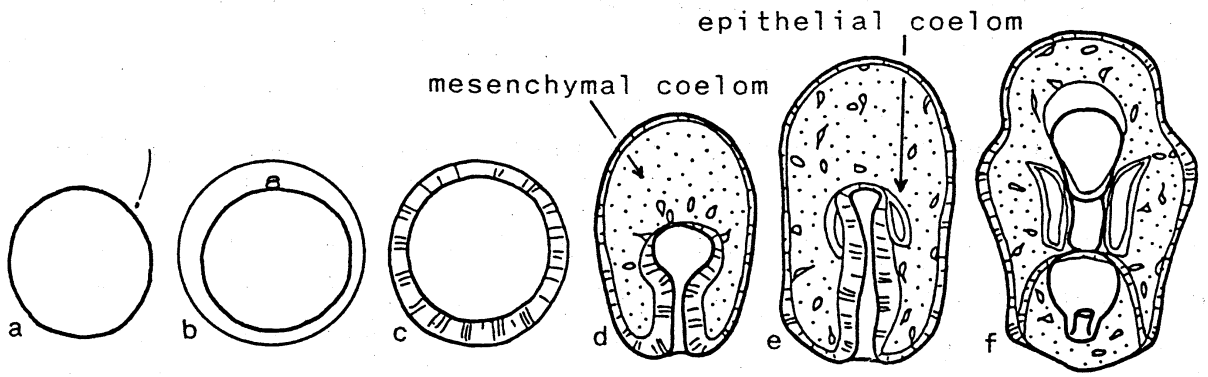

Figure 5 The process of ontogenesis of starfish

a: gametes (haploid cells) b: fertilized egg (diploid cell) c: blastula (epithelium) d: mesenchyme gastrula (mesenchymal coelom) e: very late gastrula (epithelial coelom) f: bipinnaria

7. ANALYSIS OF HIERARCHIC RELATIONSHIP IN REFERENCE TO "CRITERION 1"

From equations (1) and (2), we obtain

MITOSIS = AMITOSIS + (mitotic machinery) ..... (6)

Also from equations (1) and (4), we know that

MEIOSIS = AMITOSIS + (synamptonemal events)
+ (mitotic machinery) ..... (7)

Further, from equations (6) and (7), we obtain

MEIOSIS = MITOSIS + (synaptonemal events) ..... (8)

From equations (3) and (8), it follows that

SEXUAL REPRODUCTION = (conjugation) + MITOSIS
+ (synaptonemal events) ..... (9)

Finally, from equations (6), (9) and (5), it is concluded that

AMITOSIS ⊂ MITOSIS ⊂ SEXUAL REPRODUCTION ⊂ ONTOGENESIS.

The "relation of inclusion" thus fulfilled, we are now prepared to check the functional aspect of these methods to find out whether they possess any new function which is not found in the lower method.

8. ANALYSIS OF FUNCTION IN REFERENCE TO "CRITERION 2"

The function of AMITOSIS is to "accurately distribute a single, replicated, circular-DNA molecule between two daughter cells". That of MITOSIS, on the other hand, is to "do the same with multiple numbers of replicated, linear-DNA molecules". The latter function is obviously impossible to carry out by the mechanism of amitosis. We can tell from this fact that the two functions are not the same, and that the latter is more complex. Mitosis, therefore, is a function newly acquired at the eukaryotic stage of evolution.

The function of meiosis is to "accurately sort out two sets of DNA molecules from a completely mingled pool of DNA molecules". Synaptonemal events comprise the mechanism for this sorting out. Taking advantage of the close contact of DNA strands within the synaptonemal complex, repairing and recombination of DNA fragments between two DNA molecules are systematically incorporated (Hotta and Stern, 1976, 1981; Hotta *et al.*, 1985; von Wettstein *et al.*, 1984). Sexual reproduction as a whole, as mentioned earlier, comprises a mechanism by which "an eukaryotic cell switches its body structure between two levels of complexity, i.e., haploid and diploid, without losing its identity" (Dan-Sohkawa, 1993). Both the "sorting out" and the "switching" function of sexual reproduction is completely new to the living world at the diploid stage of evolution.

Embryonic development is a mechanism for "stepwisely build-

ing a multicellular body" from haploid level to whatever complexity the adult form belongs. This stepwise switching of body complexity is considered to be an extension of the "switching" ability of the diploid cell to multicellular levels.

Accordingly, it is concluded that each of the four reproductive methods have their own, unique function that is not found in the lower, constituent methods.

9. CONCLUSION

All four reproductive methods discussed above, i.e., AMITOSIS, MITOSIS, SEXUAL REPRODUCTION and ONTOGENESIS, are shown to fulfill the two criteria for judging hierarchic relationship between different entities, which I have set at the beginning of this paper. It is concluded, therefore, that they are truly functional entities comprising a hierarchic order of consecutively more complex structure and function of reproduction. This fact implies that, not only the structural aspect of living organisms (Dan-Sohkawa, 1992, 1993; Taylor, 1979), but also some of their functional aspects are constructed in a hierarchic manner.

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