MALE PRONUCLEUS

The male pronucleus is a transient organelle of the fertilized egg. This is made clear from a consideration of its composition and origin.

The pronucleus is bounded by a structure which has all the morphological features of a regular nuclear envelope, including pores ( ). The contents are mainly constituted by chromatin which is made up of sperm DNA packed with egg histones.

Important functions such as DNA replication ( ) and transcription have been located within the pronucleus. This means that the organelle contains a variety of enzymes, synthesized in the egg cytoplasm.( )

As the sperm head enters the egg, the nuclear envelope becomes almost entirely disintegrated ( ). It is well known for ordinary mitoses that this process involves phosphorylation of lamins with participation of Cd2, a protein of the cyclin group (Smith and Wood) The chromatin is thus brought into direct contact with the egg cytoplasm. Morphologically a decondensation of the chromatin is observed ( ), while the biochemical counterpart is a reduction of disulphide bonds and a massive replacement of the sperm protamines by egg histones.( ). Just before the formation of the pronucleus a recondensation of chromatin takes place.

The growth of the pronucleus seems to be associated with egg cytoplasm factors (Yanamigachi). and is followed by DNA synthesis and chromosome formation ( ). The latter phenomena are known to require a protein complement, especially cyclins (Smith and Wood) which are likely to be provided by the egg cytoplasm. It has been shown that mature eggs contain a cytoplasmic factor (MPF) which when acting upon somatic cells determines formation of metaphase chromosomes and when acting upon sperm heads brings about the formation of pronuclei ( ).

The pronuclear envelope becomes formed mainly from endoplasmic reticulum membranes (Yanamigachi).

It is thus clear that the majority of the components of the late pronucleus are formed or incorporated after fertilization. Only one half of the DNA could rightly be said to have been introduced with the sperm head.

The formation and evolution of the pronucleus is thus a complex morphogenetic process involving interaction between components of both gametes and taking place prior to any zygote gene expression. It can be considered as a morphogenetic pathway from the moment that it is a sequence of predictable steps, which may be thought of as the expression of an orderly dynamic dynamic system.

This latter statement deserves a detailed consideration because of its theoretical biological implications.

It is a matter of common knowledge **a)** that every organism follows a "prescribed" robust(16), developmental pathway. This means that its state at any time point within its life course <u>can be predicted</u> with reasonable accuracy; and **b)** that the states along this pathway take place within a discrete unit of living matter, i.e.

within a space separated from the environment by a well defined physical boundary.

A.- The "pathway" requirement has some interesting implications.

From a thermodynamic point of view, organisms may be thought of as chemical reactors, which interact with the environment exchanging matter and energy whose constant influx maintains the chemical components at concentrations which are far removed from their points of equilibrium.

Two very distinctive processes in the life of organisms are body growth and the replacement of the component molecules by turn over. These are made possible by networks of autocatalytic reactions, i.e. chemical reactions in which the reactants appear as products. An elementary instance could be written down as:

$$A + B \qquad 2A+C$$

It has been shown that autocatalytic systems exhibit a spontaneous tendency to bifurcation in the phase space and to symmetry breaking, in other words to selforganization (36,37). In ordinary physicochemical systems however, these changes show none of the stability and self-regulation which are prominent in living systems.

The latter characteristics have been aptly summarized by Saunders (40) referring to embryonic development: "One of the most characteristic properties of the developmental process is that it is stable. What is stable is not the state of the embryo at any one time but its pathway of development."

It seems reasonable to think that a stable trajectory of development corresponds to underlying orderly dynamics. By this is meant one in which subsequent states are predictable from those preceding them by the application of some simple set of rules. A more rigorous way of looking at it would be that it is a sequence of states which can be univocally described by the recurrence of a number of states which are shorter than the sequence itself. Since non-orderly sequences should be random, this definition bears some analogy with the one proposed by Chaitin for random numbers:a number is random if there is no program for calculating the number which is shorter than the length of the number itself(Casti vol II, p.316). This amounts to say that an orderly dynamics enters some cyclic attractor i.e. it goes through the same set of states over and over again. Cyclic attractors are a regular representation of steady state dynamics. Interesting biological analogies have been proposed for these attractors. For Kaufmann (Origins of Order) the diverse cell types in an organism might be considered as the expression of attractor cycles of the genome network.

It might therefore be asked under what conditions might a chemical reactor exhibit such dynamics. An interesting insight into this question is given by the analogy with Boolean dynamical networks (24,25,45,46).

A very simple yet instructive instance is shown in Figures 1, 2 and 3. In Fig. 1 a "string" of "cells" is portrayed each of which may find itself either in an "on" or an "off" state. The state of each cell at t=m depends exclusively on the state of its two neighbors at t=m-1. In the case under consideration the system follows a very simple "exclusive or" rule. This means that Ci at t=m will be "on" only in the case that either C i-1 or C i+1 was "on" at t=m-1. If on the contrary C i-1 and C i+1 were both "on" or both "off" at t=m-1 then Ci will be "off" at t=m.

The analogy is obvious with the model of a genome where gene products would act exclusively on the neighboring genes according to an "exclusive or" rule. (24)

However at this moment I want to stress a different view of the model. Figure 2A shows the result of iteration of the simple rule explained. It can be seen that a very definite pattern develops, which if followed long enough will repeat itself which may be interpreted by saying that the trajectory of the system entered the basin of a cyclic attractor. This behaviour exhibits a remarkable stability. Figure 2B shows the case of a more complex set of rules with the same initial conditions. (For a detailed discussion of the behaviour of this kind of systems, see the above mentioned references).

A close consideration of this model throws some light on the significance of orderly dynamics in organic development. Even the apparent simplicity of the "exclusive or" rule is quite deceptive. The very fact that any "cell" can be influenced only by two others suggests a high selectivity of the interaction rules. If the "cells" are thought of as reacting chemical species, this means that there is a high specificity of reactions , each of the chemical species present being able to react only with another two. Such a behaviour might be expected for instance of enzymes whose chemical configuration allows them to take part in a very limited number of different reactions. As mentioned below this may be expressed by saying that each of the "cells" forming the string represents a molecule with high informational content .

The evolution of complex systems is quite sensitive to initial conditions. On the other hand its behaviour is remarkably stable in face of many isolated changes even though some intercurrent alterations may become amplified in the course of its evolution.

A counterexample will render the meaning clearer. Instead of having only two "cells" determine the state of each member of the string, one may define a different "wiring" whereby a large number (in the extreme, the totality) of the "cells" have influence on the behaviour of each one. In this limit all "cells" are equivalent to one another i.e. they have a low informational content. In this case (K=N in the symbolism employed by Kauffman) , instead of orderly trajectories, the system will exhibit chaotic behaviour (24,25).

It is interesting to speculate upon the nature of the chemical species that would be likely to become involved in orderly dynamics. The main components of organisms are macromolecules, mainly nucleic acids, proteins and polysaccharides. These biopolymers are formed by linear arrays of monomeric units whose number and sequence determine the functional properties of the polymer. An enzyme for instance depends critically on the aminoacid sequence at the active site and other accessory emplacements, such as the allosteric site. If the polymers be taken to be a sequence of symbols, there is a definite number of yes/no decisions which are necessary to establish unambiguously a particular sequence. In this sense biological macromolecules have a high information content <u>with regard to the particular function they perform</u> (see 10). As a consequence they will be likely to be involved only in a limited number of reactions. This was expressed by Kauffman as follows: "A molecular reaction net of high specificity *is* a net of low connectivity . High specificity appears necessary both for precision of product formation, and to yield a system whose global chemical oscillatory behavior is brief and stable" (25).

This goes to suggest that the presence of stable (homeorhetic) predictable trajectories in the course of the life of an organism may be regarded as expression of the fact that this system is a complex non-linear dynamical system made up mainly of molecules with high informational content. Therefore a stable pathway of development might be thought of as a direct consequence of the physicochemical features of the system. Conversely it may be said that its physicochemical nature requires that the diachronical dimension be considered essential for the characterization of an organism at any moment of its lifetime.

This approach is quite generic. It applies equally to a single cell and to a multicellular organism.

B.- The "discrete unit" condition means that an organism is separated from the environment by a well defined physical boundary. This is obvious for a fully developed organism. In the case of the very early embryo, the boundary is built up both by the the plasma membrane and the zona pellucida until "hatching" time, under conditions which are briefly discussed below. (6, 9 17)

It is proposed here that the process of embryonic development starts in time at a singular point marked by the penetration of the sperm. From this moment onward a stable predictable pathway proceeds, which appears to be conditioned by the interaction of biomolecules originated both in the egg and in ths sperm, which engage into a highly coordinated trajectory. This behaviour is clearly present one or two cell cycles before zygote genome begins to be expressed in force. However in most of the phenomena concerned, there is a close intermingling of components derived from both the gametes. This amounts to say that beginning at the very moment of fertilization, the egg membrane encloses a dynamic self-organizing system that follows a clearly established developmental pathway.