A Theoretical Study on the Cell Differentiation Forming Stem Cells in Higher Animals

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Abstract

The recent genome sequencing of multicellular diploid eukaryotes reveals an enlarged repertoire of protein genes for signal transmission but it is still difficult to elucidate the network of signal transmission to drive the life cycle of such an eukaryote only from biochemical and genetic studies. In the present paper, a theoretical study is carried out for the cell differentiation, the formation of stem cells and the growth from a child to the adult in the higher animal. With the intercellular and intracellular signal transmission in mind, the cell differentiation is theoretically derived from the process by the transition of proliferated cells from proliferation mode to differentiation mode and by both the long-range interaction between distinctive types of cells and the short-range interaction between the same types of cells. As the hierarchy of cell differentiation is advanced, the original types of self-reproducible cells are replaced by the self-reproducible cells returned from the cells differentiated already. The latter type of self-reproducible cells are marked with the signal specific to the preceding differentiation and become the stem cells for the next stage of cell differentiation. This situation is realized under the condition that the differentiation of cells occurs immediately after their proliferation in the development. The presence of stem cells in the respective lineages of differentiated cells strongly suggests another signal transmission for the growth of a child to a definite size of adult that the proliferation of stem cells in one lineage is activated by the signal from the differentiated cells in the other lineage(s) and is suppressed by the signal from the differentiated cells in its own lineage. This style of signal transmission also explains the metamorphosis and maturation of germ cells in higher animals.

Keywords: Development; Growth Curve; Hormones; Metamorphosis; Signal Transmission

Introduction

The life of a higher animal consists of the proliferation of a fertilized egg by mitosis, the differentiation of proliferated cells to form organs and tissues, growth to adult, production of germ cells by meiosis and death. This curious phenomenon has been studies from several approaches. The first approach is the experimental embryology that inquires into the initiation of cell differentiation by cutting an embryo and/or transplanting some part to other regions in the embryo at various stages of development. This approach finds in the newt embryo that the dorsal vegetative guadrant of gastrula becomes the first organizer to induce the other types of cells [1,2]. Similar approach is then extended to the embryo from asymmetrical *Xenopus* egg [3,4], to *Drosophila* embryo by focusing on surrounding cells [5,6], and to the embryo from symmetrical mammalian egg [7,8]. Such approach also reveals a common pattern of forming body plan that the cells proliferated from a fertilized egg first differentiate into the ectoderm and the endoderm and then between them, the mesoderm appears.

The DNA replication, chromosome segregation and auxiliary molecular machine upon the mitosis and meiosis are visualized by optical microscopy of living cells and by electron microscopy of fixed and stained cells [9]. The biochemical studies find that the mitosis is triggered by the activity of MPF kinase to increase the phosphorylation of specific proteins [10,11], while the Map kinase is reported to signal the pathway in oocyte meiosis [12,13]. A close relation of cell division with phosphorylation is also reported [14].

The recent gene and genome sequencing is revealing a notable feature that most cells of a multicellular diploid eukaryote each carry the enlarged repertoire of protein genes for intercellular and intracellular signal transmission such as receptors and kinases as well as for cell adhesion [15-18]. Moreover, many kinds of proteins including transcriptional regulators carry long stretches of serine and threonine residues, which are known to be the sites for phosphorylation [9] and glycosylation [19] to change the activity and specificity of the proteins. The transmission pathways of phosphorylation signal are partly followed experimentally from the receptor accepted ligand to transcriptional regulators in some examples of differentiated cells [9], but they are not simply linear relay chains but branch to activate many interaction components that operate in parallel, forming interconnected signal network. This complexity of signal transmission   prevents the elucidation of cell behavior in a multicellular eukaryote only from the biochemical and genetic approaches.

Thus, theoretical studies are needed towards the comprehensive understanding of the cell behavior. In the previous paper [20], the basic condition for cell differentiation is theoretically formulated by the transition from proliferation mode to differentiation mode and by both the long-range interaction between distinctive types of cells and the short-range interaction between the same type of cells. In the present paper, this theoretical formulation is extended to the higher hierarchy of cell differentiation forming the stem cells in higher animals. Although the stem cells are indicated only for the renewal of corresponding lineage of differentiated cells [21], the signal transmission to stimulate and suppress the proliferation of stem cells will be also proposed to explain the growth curve of a higher animal including the metamorphosis and the maturation of germ cells.

Theoretical Formulation of Cell Differentiation

Several stages of cell differentiation forming the stem cells are schematically shown in Figure 1. Along this line, the cell differentiation and the formation of stem cells will be theoretically formulated in this and succeeding sections,
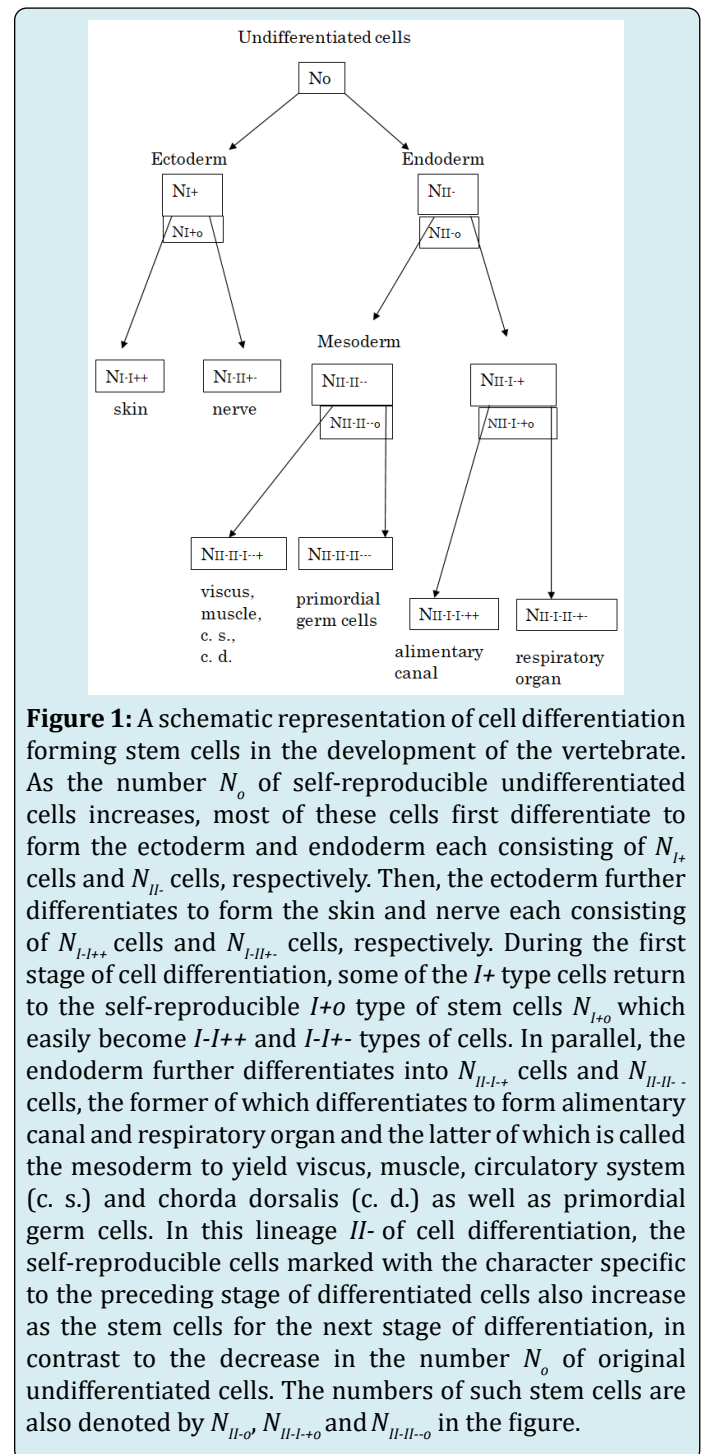
respectively.

Figure 1: A schematic representation of cell differentiation forming stem cells in the development of the vertebrate. As the number N_o of self-reproducible undifferentiated cells increases, most of these cells first differentiate to form the ectoderm and endoderm each consisting of N_{I+} cells and N_{II-} cells, respectively. Then, the ectoderm further differentiates to form the skin and nerve each consisting of N_{I-I++} cells and N_{I-II+-} cells, respectively. During the first stage of cell differentiation, some of the $I+$ type cells return to the self-reproducible $I+o$ type of stem cells N_{I+o} which easily become $I-I++$ and $I-I+-$ types of cells. In parallel, the endoderm further differentiates into N_{II-I-+} cells and $N_{II-II--}$ cells, the former of which differentiates to form alimentary canal and respiratory organ and the latter of which is called the mesoderm to yield viscus, muscle, circulatory system (c. s.) and chorda dorsalis (c. d.) as well as primordial germ cells. In this lineage $II-$ of cell differentiation, the self-reproducible cells marked with the character specific to the preceding stage of differentiated cells also increase as the stem cells for the next stage of differentiation, in contrast to the decrease in the number N_o of original undifferentiated cells. The numbers of such stem cells are also denoted by N_{II-o}, $N_{II-I-+o}$ and $N_{II-II--o}$ in the figure.

When the number of proliferated cells becomes N, the first stage of cell differentiation into two types, + and -, occurs. This process of cell differentiation proceeds more rapidly than the proliferation, and can be analyzed considering that the total number N of cells is almost constant. As shown previously [20], the mathematical formulation of

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cell differentiation process starts from the master equation of the probability $P(N_{I+}, N_{I-}, N_{II+}, N_{II-}, N_o; t)$, with which we find N_{I+} cells of + type and N_{I-} cells of - type in region I, N_{II+} cells of + type and N_{II-} cells of - type in region II, and N_o self-reproducible undifferentiated cells at time t. The master equation expresses the time-change in this probability due to the following terms. The first kind of terms are transition probability $e^{\alpha I}$ from an undifferentiated cell to + or - type of cell in the region I and the transition probability $e^{\alpha II}$ from an undifferentiated cell to + or - type of cells in the region II. The probabilities of reverse transition from these differentiated cells to the undifferentiated cells are denoted by $e^{-\alpha I}$ and $e^{-\alpha II}$, respectively. Using the Ising model [22] that the + type of cell takes the value of +1 and the - type of cell takes the value of - 1, the second kind of terms express that the cells in the same region tend to take the same type by the short-range interaction with the strength β_I in the region I and that with the strength β_{II} in the region II, while the cells in the region I tend to take the type different from the type of cells in the region II by the long-range interaction with the strength γ.

When the total number of cells is N (= $N_{I+} + N_{I-} + N_{II+} + N_{II-} + N_o$), we focus on the following four quantities defined by

$$Y_I \equiv \frac{N_{I+} + N_{I-}}{N}, Y_I \equiv \frac{N_{I+} + N_{I-}}{N}, y_I \equiv \frac{N_{I+} - N_{I-}}{N}, y_I \equiv \frac{N_{I+} - N_{I-}}{N}$$

(1)

Then, the master equation of the probability $P(N_{I+}, N_{I-}, N_{II+}, N_{II-}, N_o; t)$ is changed into the equation of probability density function $p(Y_I, Y_{II}, y_I, y_{II}; t)$. If the N is sufficiently large, it is obtained from the equation of probability density function that the most probable values of above four quantities mainly obey the following time-change equations [20].

$$\frac{d}{dt}Y_I = -Y_I e^{-\alpha I}\cosh(\beta_I y_I - \gamma y_{II}) + 2(1 - Y_I - Y_{II})e^{\alpha I}\cosh(\beta_I y_I - \gamma y_{II})$$

(2)

$$\frac{d}{dt}Y_{II} = -Y_{II} e^{-\alpha II}\cosh(\beta_{II} y_{II} - \gamma y_I) + 2(1 - Y_I - Y_{II})e^{\alpha II}\cosh(\beta_{II} y_{II} - \gamma y_I)$$

(3)

$$\frac{d}{dt}y_I = 4Y_I \sinh(\beta_I y_I - \gamma y_{II}) - 4y_I \cosh(\beta_I y_I - \gamma y_{II})$$ (4)

$$\frac{d}{dt}y_{II} = 4Y_{II} \sinh(\beta_{II} y_{II} - \gamma y_I) - 4y_{II} \cosh(\beta_{II} y_{II} - \gamma y_I)$$ (5)

Here, the following relation also holds

$$N(1 - Y_I - Y_{II}) = N_o$$ (6)

The set of Equations (2-5) shows the behavior of cells towards the following differentiation. For the positive values of α_I and α_{II}, Y_I and Y_{II} direct to the stationary values Y_{Ist} and Y_{IIst}, which satisfy $dY_I/dt = 0$ and $dY_{II}/dt = 0$ in Equations (2) and (3), respectively. Then, the following relations hold between Y_{Ist} and Y_{IIst}:

$$Y_{Ist} = 2(1 - Y_{Ist} - Y_{IIst})e^{2\alpha I}$$ (7)

$$Y_{IIst} = 2(1 - Y_{Ist} - Y_{IIst})e^{2\alpha II}$$ (8)

From Equations (7) and (8), Y_{Ist} and Y_{IIst} are expressed in terms of the transition probabilities $e^{\alpha I}$ and $e^{\alpha II}$ by the following way.

$$Y_{Ist} = \frac{2e^{2\alpha I}}{1 + 2e^{2\alpha I} + 2e^{2\alpha II}}$$ (9)

$$Y_{IIst} = \frac{2e^{2\alpha II}}{1 + 2e^{2\alpha I} + 2e^{2\varepsilon II}}$$ (10)

The relation between Y_{Ist} and y_I and that between Y_{IIst} and y_{II} at the stationary state are obtained from Equations (4) and (5), respectively, by setting $dy_I/dt = 0$ and $dy_{II}/dt = 0$. That is,

$$y_I = Y_{Ist} \tanh(\beta_I y_I - \gamma y_{II})$$ (11)

$$y_{II} = Y_{IIst} \tanh(\beta_{II} y_{II} - \gamma y_I)$$ (12)

The solution of y_I and y_{II} satisfying Equations (11) and (12) are estimated as y_{Ist} and y_{IIst}, respectively, by the graphical method shown in Figure 2, indicating the cell differentiation that y_{Ist} becomes a positive value when y_{IIst} takes a negative value. In particular, y_{Ist} and y_{IIst} become nearly equal to Y_{Ist} and $-Y_{IIst}$, that is, to N_{I+}/N and $-N_{II-}/N$, respectively, when the interaction strength, β_I, $\beta_{II} > 0$ and $\gamma > 0$, is sufficiently strong.

The long-range interaction is probably caused by the pair-wise ligand-receptor relationship; the $I+$ type of cell secretes the ligand l_{I+} which is accepted by the receptor r_{II-} on the surface of $II-$ type of cell, while the $II-$ type of cell secretes the ligand l_{II-} to be accepted by the receptor r_{I+} on the surface of $I+$ type cell. The receptor r_{I+} accepted the ligand l_{II-} induces the expression of the gene(s) characteristic to the + type cell through the intracellular signal transmission to the transcriptional regulator(s), and the receptor r_{II-} accepted the ligand l_{I+} induces the expression of the gene(s) characteristic to the - type cell. The short-range interaction probably arises from the direct contact between the same type of cells to stabilize the intracellular signal transmission induced by the long-range interaction through the cytoskeleton.
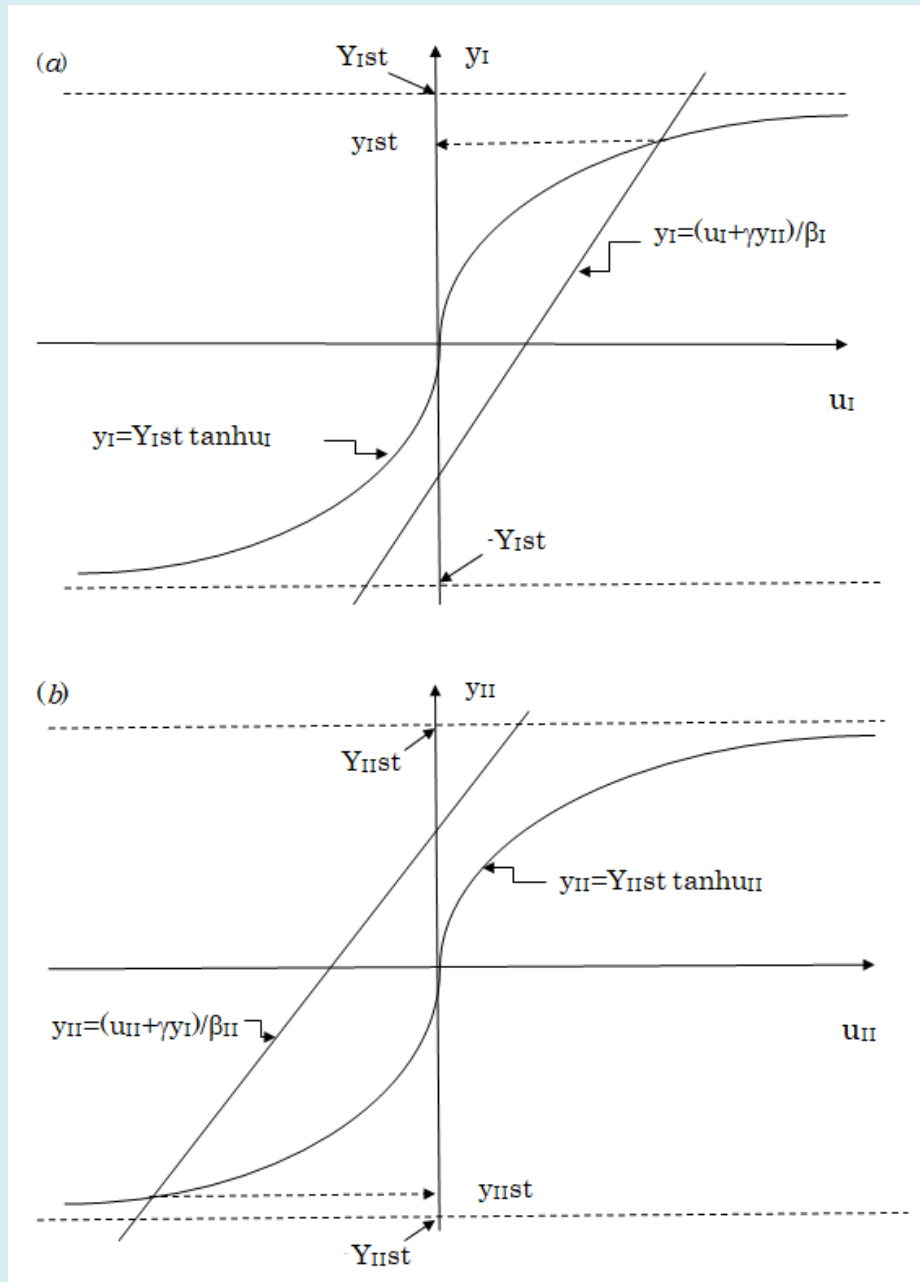
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Figure 2: The graphical procedure to estimate the values of y_I and y_{II} satisfying Equations (11) and (12). (*a*) When a new variable u_I is introduced by $u_I = \beta_I y_I - \gamma y_{II}$, Equation (11) is rewritten into $y_I = Y_{Ist} \tanh u_I$. The values of y_I are plotted against the values of u_I according to the first equation under a constant value of y_{II} and to the second equation. The value of y_{Ist} satisfying Equation (11) is obtained as the ordinate of the crossing point of the straight line expressed by the first equation with the curve expressed by the second equation. This value of y_{Ist} becomes positive when the value of y_{II} is chosen to be negative. (*b*) Introducing a new variable u_{II} defined by $u_{II} = \beta_{II} y_{II} - \gamma y_I$, Equation (12) is rewritten into $y_{II} = Y_{IIst} \tanh u_{II}$. The values of y_{II} are plotted against the values of u_{II} according to these two equations under a constant value of y_I. The value of y_{IIst} satisfying Equation (12) is obtained as the ordinate of the crossing point of the straight line with the curve expressed by the above two equations. As seen in the figure, the value of y_{IIst} thus obtained becomes negative when y_I is chosen to be a positive value. This is consistent with the result of (*a*).

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At the stage when the number N_{I+} of *I+* type cells becomes larger, the *I+* type of cells further differentiate into *I-I++* and *I-II+-* types of cells. By the procedure similar to that of the first stage, the second stage of cell differentiation is mathematically expressed in the following way. In the lineage *I+*, new variables Y_{I-I}, Y_{I-II}, y_{I-I} and y_{I-II} are defined by

$$Y_{I-I} \equiv \frac{N_{I-I++} + N_{I-I+-}}{N_{I+}}, Y_{I-II} \equiv \frac{N_{I-II++} + N_{I-II+-}}{N_{I+}}$$

$$y_{I-I} \equiv \frac{N_{I-I++} - N_{I-I+-}}{N_{I+}}, y_{I-II} \equiv \frac{N_{I-II++} - N_{I-II+-}}{N_{I+}} \quad (13)$$

using the number N_{I-I++} of ++type cells and the number N_{I-I+-} of +- type cells in the region *I-I*, and the number N_{I-II++} of ++type cells and the number $N_{II-II+-}$ of +- type cells in the region *I-II*. Here, N_{I+} is the number of all cells in the lineage *I+*. The quantity N_{I+0} defined by

$$N_{I+0} = N_{I+}(1 - Y_{I-I} - Y_{I-II}) \quad (14)$$

is the number of stem cells in the lineage *I+*, which will be investigated in the next section. Y_{I-I} and Y_{I-II} are directed to the following stationary values Y_{I-Ist} and Y_{I-IIst} determined by the transition probabilities $e^{\alpha I-I}$ from *I+* type cells to *I-I++* and *I-I+-* types of cells in the *I-I* region and by the transition probability $e^{\alpha I-II}$ to *I-II++* and *I-II+-* types of cells in the *I-II* region.

$$Y_{I-Ist} = \frac{2e^{2\alpha I-I}}{1 + 2e^{2\alpha I-I} + 2e^{2\alpha I-II}} \quad (15)$$

$$Y_{I-IIst} = \frac{2e^{2\alpha I-II}}{1 + 2e^{2\alpha I-I} + 2e^{2\alpha I-II}} \quad (16)$$

The fractions y_{I-I} and y_{I-II} in the regions *I-I* and *I-II* are determined by the long-range interaction strength $\gamma_{I-I;I-II}$ between the cells in the region *I-I* and those in the region *I-II* as well as by the short-range interaction strength β_{I-I} and β_{I-II} in the respective regions, as follows .

$$y_{I-I} = Y_{I-Ist} \tanh(\beta_{I-I} y_{I-I} - \gamma_{I-I;I-II} y_{I-II}) \quad (17)$$

$$y_{I-II} = Y_{I-IIst} \tanh(\beta_{I-II} y_{I-II} - \gamma_{I-I;I-II} y_{I-I}) \quad (18)$$

By the graphical procedure similar to Equations (11) and (12), the cell differentiation of $y_{I-Ist} \sim N_{I-I++}/N_{I+}$ and $y_{I-IIst} \sim -N_{I-II+-}/N_{I+}$ are obtained from Equations (17) and (18).

In parallel, *II-* type of cells also differentiate into *II-I-+*

and *II-II - -* types of cells according to the following set of equations.

$$Y_{II-I} \equiv \frac{N_{II-I-+} + N_{II-I--}}{N_{II-}}, Y_{II-II} \equiv \frac{N_{II-II-+} + N_{II-II--}}{N_{II-}}$$

$$y_{II-I} \equiv \frac{N_{II-I-+} - N_{II-I--}}{N_{II-}}, y_{II-II} \equiv \frac{N_{II-II-+} - N_{II-II--}}{N_{II-}} \quad (19)$$

$$N_{II-0} = N_{II-}(1 - Y_{II-I} - Y_{II-II}) \quad (20)$$

$$Y_{II-Ist} = \frac{2e^{2\alpha II-I}}{1 + 2e^{2\alpha II-I} + 2e^{2\alpha II-II}} \quad (21)$$

$$Y_{II-IIst} = \frac{2e^{2\alpha II-II}}{1 + 2e^{2\alpha II-I} + 2e^{2\alpha II-II}} \quad (22)$$

and

$$y_{II-I} = Y_{II-Ist} \tanh(\beta_{II-I} y_{II-I} - \gamma_{II-I;II-II} y_{II-II}) \quad (23)$$

$$y_{II-II} = Y_{II-IIst} \tanh(\beta_{II-II} y_{II-II} - \gamma_{II-I;II-II} y_{II-I}) \quad (24)$$

The values of y_{II-Ist} and $y_{II-IIst}$ satisfying Equations (23) and (24) are also obtained by the graphical procedure.

In this way, the first and second stages of cell differentiation shown in Figure 1 are reproduced mathematically. The mathematical expression for the succeeding stages of cell differentiation are also possible as the extension of the present formulation.

Formation and Property of Stem Cells

In this section, the changes in self-reproducible cells will be theoretically investigated in a longer time scale than the process of cell differentiation at each stage. During the first stage, the number N_{I+} of + type cells in the region *I* changes with time in the following relation to the number N_o of self-reproducing undifferentiated cells.

$$\frac{d}{dt} N_{I+} = e^{\alpha I} N_o - e^{-\alpha I} N_{I+} \quad (25)$$

In the same way, the number N_{II-} of - type cells in the region *II* changes with time by the following equation.

$$\frac{d}{dt} N_{II-} = e^{\alpha II} N_o - e^{-\alpha II} N_{II-} \quad (26)$$

Meanwhile, the number N_o of undifferentiated cells also changes with time according to the following equation.

$$\frac{d}{dt}N_o = \lambda_o N_o - e^{\alpha I}N_o - e^{\alpha II}N_o \quad (27)$$

Here, λ_o is the proliferation rate of undifferentiated cells. By formally integrating Equation (25) with respect to time t, the following expression of time dependency of N_{I+} is obtained.

$$N_{I+}(t) = \exp\{-e^{-\alpha I}(t-t_0)\}$$

$$\int_{t0}^{t} e^{\alpha I}N_o(\tau)\exp\{e^{-\alpha I}(\tau-t_0)\}d\tau + N_{I+}(t_0)] \quad (28)$$

From Equation (27), the time dependence of $N_o(t)$ is expressed as

$$N_o(t) = N_o(t_0)\exp\{\lambda(t-t_0)\} \quad (29)$$

Here,

$$\lambda = \lambda_o - e^{\alpha I} - e^{\alpha II} \quad (30)$$

For the formation of stem cells, it is necessary for λ to be nearly equal to zero, that is, the proliferated cells immediately change to the differentiation mode. Then, $N_o(t)$ in the integral of Equation (28) is regarded as almost constant N_o independent of time within the range of $t_o < \tau < t$, and this equation is reduced to

$$N_{I+}(t) \approx e^{2\alpha I}N_o[1 - \exp\{-e^{-\alpha I}(t-t_0)\}]$$
$$+ N_o\exp\{-e^{-\alpha I}(t-t_0)\} \quad (31)$$

Thus, $N_{I+}(t)$ increases from N_o to $e^{2\alpha I}N_o$ with time. During this period, the number N_{I+o} of self-reproducible cells having returned from $I+$ type of cells increases first according to

$$\frac{d}{dt}N_{I+o}(t) = e^{-\varepsilon I}N_{I+}(0) = e^{-\alpha I}N_o \quad (32)$$

but finally increases according to the equation.

$$\frac{d}{dt}N_{I+o}(t) = e^{-\alpha I}N_{I+}(t) = e^{\alpha I}N_o \quad (33)$$

In the similar way, the number N_{II-o} of self-reproducible cells having returned from the $II-$ type cells increases finally according to the equation.

$$\frac{d}{dt}N_{II-o}(t) = e^{\alpha II}N_o \quad (34)$$

Thus, the numbers N_{I+o} and N_{II-o} becomes larger than N_o at the later period of the first stage of cell differentiation. If the I_{+o} and II_{-o} types of self-reproducible cells still carry the receptor-ligands $r_{I+}l_{II-}$ and $r_{II-}l_{1+}$ as well as the corresponding intracellular signal transmitters, respectively, they are easier

to become the second stage of differentiated cells, *I-I++* and *I-II+-* types of cells, and *II-I-+* and *II-II--* types of cells, respectively, than the original self-reproducible cells N_o. Thus, N_{I+o} cells and N_{II-o} cells correspond to the stem cells for the second stage of cell differentiation.

At the second stage of cell differentiation, the term of $-(e^{\alpha I-I}+e^{\alpha I-II})N_{I+o}$ is added to the right hand side of Equation (33) and the increase in N_{I+o} is stopped. Instead, new types of stem cells having returned from *I-I++* and *I-II+-* types of cells increase in their numbers N_{I-I++o} and $N_{I-II+-o}$ by the following way

$$\frac{d}{dt}N_{I-I++o}(t) = e^{\alpha I-I}N_{I+o} \quad (35)$$

$$\frac{d}{dt}N_{I-II+-o}(t) = e^{\alpha I-II}N_{I+o} \quad (36)$$

In the same way, the numbers $N_{II-I-+o}$ and $N_{II-II--o}$ of self-reproducible stem cells increase with time according to the following equations

$$\frac{d}{dt}N_{II-I-+o}(t) = e^{\alpha II-I}N_{II-o} \quad (37)$$

$$\frac{d}{dt}N_{II-II--o}(t) = e^{\alpha II-II}N_{II-o} \quad (38)$$

The stem cells formed in this way are advantageous to advance the cell differentiation towards the higher hierarchy, although the regeneration of a lost organ containing the stem cells becomes harder.

Regulation and Control on the Proliferation Rates of Stem Cells in Different Lineages

After the development of an embryo, the born child grows to a definite size of adult, taking preys. During this growth, the organs and tissues are enlarged by the proliferation of stem cells in the respective lineages. Thus, it is necessary to consider the problem how the proliferation of stem cells in different lineages is coordinately regulated and controlled to form a definite size of adult suitable for preying activity and breeding. The following two cases are considerable for this problem, alternatively. (i) The proliferation rate of stem cells in one lineage is raised by the signal from the differentiated cells in other lineage(s) and it is suppressed by the signal from the differentiated cells in its own lineage. (ii) The proliferation rate of stem cells in one lineage is raised by the signal from the differentiated cells in its own lineage and is suppressed by the signal from the differentiated cells in other lineage(s).

It is a simple model scheme for the case (i) that the differentiated cells in the i-th lineage stimulate the proliferation of stem cells in $(i+1)$-th lineage ($i = 1, 2, ...,k$ and $k+1=1$) and the proliferation of stem cells in each lineage is suppressed by the differentiated cells in its own lineage. Mathematically, this is expressed by the following set of equations concerning the time change in the numbers $N_1, N_2 ......,$ and N_k of differentiated cells in the lineages $1, 2, ...,$ and k.

$$\frac{d}{d}N_1 = a_{1k}N_k - r_1 N_1^{m1} \quad (39)$$

$$\frac{d}{dt}N_2 = a_{21}N_1 - r_2 N_2^{m2} \quad (40)$$

$$........................$$

$$\frac{d}{dt}N_k = a_{kk-1}N_{k-1} - r_k N_k^{mk} \quad (41)$$

Here, a_{ii+1}'s, r_i's and m_i's are assumed to be constant for simplicity. Then, the numbers of differentiated cells in all lineages increase with time towards a stable stationary state, in which N_{ist}'s are written in terms of $a_{i,i-1}$'s, r_i's and m_i's as follows.

$$N_{1st} = \{(\frac{a_{21}}{r_2})(\frac{a_{32}}{r_3})^{m2}(\frac{a_{43}}{r_4})^{m2m3}..........(\frac{a_{1k}}{r_1})^{m2m3.......mk-1mk}\}^{1/(m1m2m3.........mk-1)}$$

$$(42)$$

$$N_{2st} = \{(\frac{a_{32}}{r_3})(\frac{a_{43}}{r_4})^{m3}(\frac{a_{54}}{r_5})^{m3m4}........(\frac{a_{21}}{r_2})^{m3m4.......mkm1}\}^{1/(m1m2m3.........mk-1)}$$

$$(43)$$

$$...$$

$$\frac{d}{dt}N_{kst} = \{(\frac{a_{1k}}{r_1})(\frac{a_{21}}{r_2})^{m1}(\frac{a_{32}}{r_3})^{m1m2}..........(\frac{a_{kk-1}}{r_k})^{m1m2m3.......mk-1}\}^{1/m1m2m3.........mk-1)}$$

$$(44)$$

As seen from this result, $a_{ii+1} > r_i$ and $m_i > 1$ are necessary for N_{ist}'s to represent an enlarged body size of the adult. The stability of this stationary state is illustrated in Figure 3 for the simplest model of $k = 2$ and $m_1 = m_2 = 2$. In the case when the values of a_{ii-1}'s, r_i's and m_i's are so adjusted as to retain the ratio of differentiated cells determined by the transition probabilities e^{α}'s and the long-range and short-range interaction strength in the development, almost the same body shape is retained during the growth from a child to the adult. On the contrary, the body shape is considerably changed during the growth, when the values of some of a_{ii-1}'s, r_i's and m_i's are changed, for example, by the secretion of hormones. The metamorphosis is an extreme case of such example, as will be discussed in the last section. At any rate, the case (i) is compatible with the growth to a definite size of adult.

In contrast to the case (i), no stable growth point is obtained for the case (ii). This result is instructive in considering the stability of a population consisting of interacting elements under birth and death process.
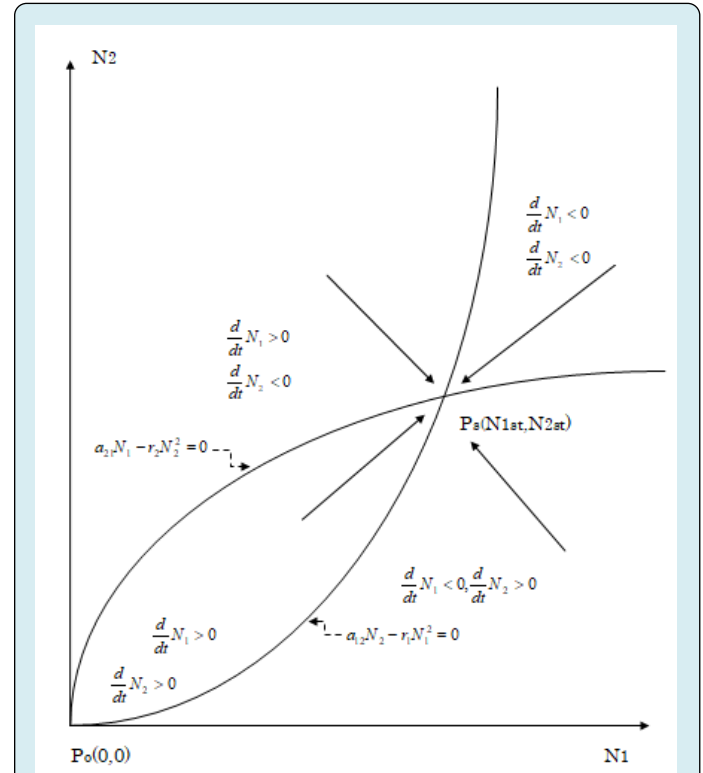

Figure 3: The simplest model for the regulation and control on the proliferation of stem cells in two lineages. The time change in the numbers N_1 and N_2 of differentiated cells in the respective lineages is illustrated according to the following set of two equations.

$$\frac{d}{dt}N_1 = a_{12}N_2 - r_1 N_1^2 \quad , \quad \frac{d}{dt}N_2 = a_{21}N_1 - r_2 N_2^2$$

The two curves each obtained by setting the right hand of each of the above equations to be zero are plotted on the plane expanded by rectangular axes N_1 and N_2. These curves have two crossing points $P_o(0, 0)$ and $P_s(N_{1st}, N_{2st})$. Here, $N_{1st} = \{(a_{21}/r_1)(a_{12}/r_2)^2\}^{1/3}$ and $N_{2st}=\{(a_{12}/r_2)(a_{21}/r_1)^2\}^{1/3}$. The positive or negative value of dN_1/dt and dN_2/dt is denoted in the four regions divided by the above two curves, and the corresponding increase or decrease in N_1 and N_2 is shown by an arrow in each region. As seen in the figure, the cell assembly consisting of N_1 and N_2 near the point P_o is directed towards the stable stationary state represented by the point $P_s(N_{1st}, N_{2st})$.

Biological Activity

The biological activity *BA* proposed previously [23] gives a useful measure for a whole state of an animal during the growth. This thermodynamic quantity is defined as follows, using the temperature *T*.

$$BA \equiv E_a(\{N_i\}, S_n(\{N_i\})) - E_s(\{N_i\}) - TS_n(\{N_i\})$$

(44)

The difference between the energy E_a acquired by the cooperative action of differentiated cells and the energy E_s stored in the set $\{N_i\}$ of differentiated cells is released as heat to compensate for the negative entropy - $S_n(\{N_i\})$ of systematization of differentiated cells, being consistent with the second law of thermodynamics. The acquired energy, which is first given from female parent but then acquired by taking preys, is mainly used to increase the numbers of differentiated cells during the growth but it is gradually accumulated to raise the biological activity (probably in the form of ATP and/or GTP molecules) as the increase in the numbers of differentiated cells slows down. The raised biological activity then stimulates the metamorphosis and the maturation of primordial germ cells through the secretion of hormones. As the animal ages, its biological activity decreases as will be discussed in the last section.

Conclusions and Discussion

In the present paper, a theoretical study is carried out for the cell behavior, mainly focusing on the cell differentiation, formation of stem cells and the regulation on the proliferation of stem cells to explain the growth from an embryo to a definite size of adult. This theory contains the signal transmission pathways still unidentified as well as experimentally identified ones. For the future identification of the former, the signal transmission indicated in the present study will be first summarized into the following four categories (A) ~ (B).

(A) Signal for the transition between the proliferation mode and the differentiation mode. Recently, a technological method to convert differentiated cells into self-reproducible undifferentiated ones is devised by injecting the four kinds of genes, *Oct3/4*, *Sox 2*, *Klf4* and *c-Mty-c* [24,25]. Originally, these genes are expressed specifically in the embryonic stem (*ES*) cell, and the products of these genes may play the role in erasing the signals for the long-range and short-range interactions and in converting α's in the transition probabilities to negative values. This also suggests the presence of genes whose products suppress the expression of the above four genes and promote the transition to the differentiation mode in response to the increase in the density of cells.

(B) The signal transmission responsible for cell differentiation. This type of signal transmission causes the long-range interaction between distinctive types of cells and the short-range interaction between the same type of cells. In addition to the identification of this signal transmission, it is also a future problem to ascertain whether the advanced form of differentiated cells, e. g. denoted by *II-II-+*, are under the influence of double pairs of long-range interaction and short-range interaction, or indicate the two steps of molecular events to express the specific gene(s), first under the interaction pair characterized by the state - and then changes under the second interaction pair characterized by the state +.

(C) Signal transmission in response to raised biological activity. The gonadotrophic hormones are known to stimulate the proliferation of primordial germ cells leading to the meiosis by the intracellular phosphorylation due to Map kinase [12,13]. The metamorphosis is also the representative example that the proliferation of stem cells and cell differentiation in one group of lineages are changed to those in another group of lineages in response to the raised biological activity. In many species of insects, most of cells forming the larva are led to programmed death or apoptosis and are used as the material and energy source for the stem cells in another group of lineages to form the adult by the action of prothoraciatrophic hormone and prothoracid hormone [26], although the adult hardly enlarges and dies soon after breading. The evolution from fish to land vertebrates can be traced in the metamorphosis from the tadpole to the frog by the action of thyroid hormone [27]. This kind of hormone also plays an important role in maintaining various organs of other land vertebrates [28]. In such land vertebrates, the metamorphosis occurs during the fetus and the signal transmission to regulate and control the proliferation of stem cells described in the fourth section takes place from the born child to the adult, while most species of fish grow without the metamorphosis.

(D) Signal transmission for raising and suppressing the proliferation rate of stem cells. Besides the hormones to cause the metamorphosis, the growth hormone synthesized in and secreted from the anterior lobe of pituitary gland [29] may play a central role in raising the proliferation rate of stem cells during the growth. Although the signal to suppress the proliferation of stem cells in its own lineage is not identified yet, the autonomic nervous system might be associated with such suppression. Anyway, the proliferation of stem cells becomes restricted to renew the cells of organs and tissues after the adult reaches a definite size.

As the renewal ability of stem cells is lowered, the senile weakness occurs leading to death. It is reasonable that the life span of an animal is regulated to be finite in this way,

because any survived organism has maintained and extended their negative entropy to acquire the material and energy from the outside by reproducing children including mutants and by the selection of them [30,31]. Even if we confine ourselves to the base change in DNA genes arising from the mis-repair of damaged bases, its change rate amounts to 10^{-9} per site per year [32,33]. Even by such point mutation, receptors, kinases and the proteins each carrying serine-threonine repeat easily change their activity and specificity. Although many of such changes are defective, some others contribute to expand the repertoire of these protein genes by occurring in the counterpart of their duplicated genes. In this sense, the signal transmission is an important factor in the evolution, especially with respect to the ratio of organs, body size, life span and the adaptation to the environment. This may explain the difference between human and chimpanzee, which diverged only about 7×10^6 years ago [34].

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