The Asymmetric Division of Cells and the Immortal Strand Hypothesis

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ABSTRACT

There are two widely accepted mechanisms for asymmetric division of cells as required for splitting a clone of stem cells into sub-clones. They are designated "intrinsic" and "extrinsic" depending upon the apparent origin of asymmetry (polarization). Here I will focus mainly on the intrinsic mechanism. Neither of these mechanisms has been proven beyond doubt experimentally and neither is known to be universally applicable. Moreover, it is not clear how either of those mechanisms would be regulated during development. In both cases, the accepted mechanisms involve induction of an asymmetry in the cytoplasm of the cell prior to cell division and only during subsequent proliferation are the genomes reprogrammed by cytoplasmic factors to a new state of differentiation. Here, I propose a completely different concept in which asymmetry is introduced into the imprinting of the chromosomes during the G2 phase after DNA synthesis by RNA-directed epigenetic marking of the newly synthesized strand of DNA. At no time is asymmetry of the cytoplasm or the surrounding cells required or involved in the reprogramming. The key is that in the second generation, the two differently imprinted strands of DNA will be templates for marking the corresponding newly synthesized strand of DNA such that when the cells divide, the original type is restored and a newly differentiated type is produced. It is noted that the DNA that retains the original parental imprinting also retains full telomere length and is not "aged" during the process, whereas the newly synthesized strands with new imprinting progressively lose telomere length generation-by-generation. The parental strand also avoids replication errors because it is always the template strand. This mechanism explains the "immortal strand hypothesis" and shows why some of the attempts to prove the "immortal strand hypothesis" produced negative results because it is the pattern of imprinting rather than the sequence of base-pairs that determines differentiation.
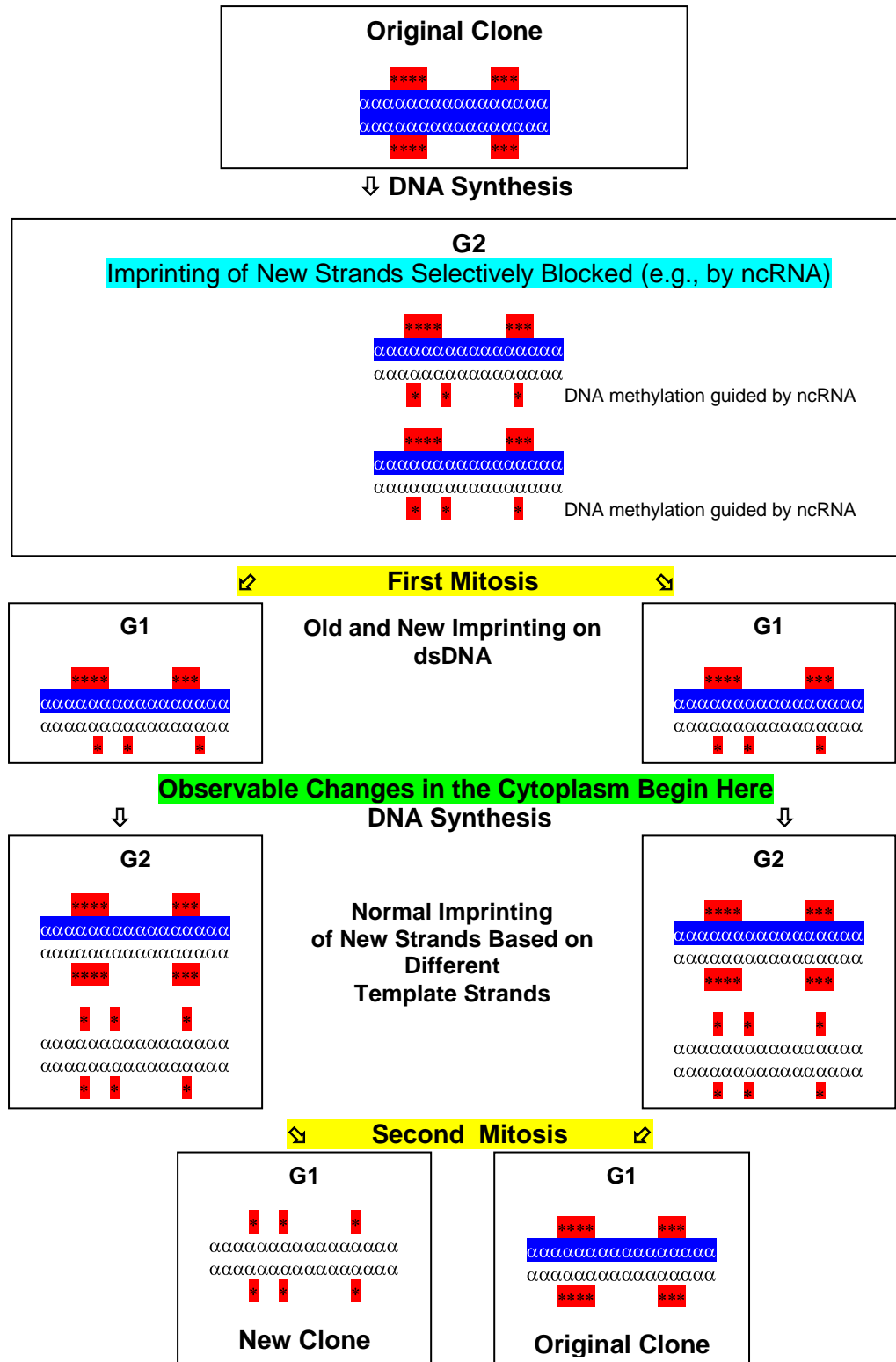
Introduction

It has long been recognized that development of complex organisms requires a reproducible pattern of differentiation of a hierarchy of stem cells starting with the zygote [1]. The branching of the original cell line into different cell lines happens repeatedly and must be done precisely at the correct generation of the cell clone to achieve the desired morphology and physiology of the complex organism. The high level of developmental fidelity observed in identical twins and among all members of the same species (produced under a wide variety of climatic, environmental, nutritional and cultural conditions) testifies to the robustness of the system. The mechanism through which asymmetric division is accomplished must be marvelously simple and must be compatible with the overall asymmetry produced in the maturing organism.

Differentiation requires progressive reprogramming of the genome by changes in the pattern of CpG methylation (which determines the pattern of histone methylation and state of chromatin condensation) [2, 3]. The important element of asymmetric division is a description of how this reprogramming occurs. This is precisely the topic frequently omitted from the conventional discussion of asymmetric division. For example, some of the major reviews of asymmetric stem cell division do not even mention it and focus instead on redistribution of proteins in the cytoplasm [4-6].

Regarding Accepted Mechanisms of Asymmetric Division

There are two generally accepted mechanism by which asymmetric stem cell division occurs [4].

Figure 1. A new two-step mechanism for asymmetric cell division.

Original Clone

⇩ **DNA Synthesis**

G2

Imprinting of New Strands Selectively Blocked (e.g., by ncRNA)

DNA methylation guided by ncRNA

DNA methylation guided by ncRNA

↯ **First Mitosis** ↯

G1   **G1**

Old and New Imprinting on dsDNA

Observable Changes in the Cytoplasm Begin Here
DNA Synthesis

G2   **G2**

Normal Imprinting
of New Strands Based on
Different
Template Strands

↯ **Second Mitosis** ↯

G1   **G1**

New Clone   **Original Clone**

They have been designated intrinsic and extrinsic. Intrinsically determined asymmetric division predominates during development and is of most interest here. It is understood that stem cell differentiation (primarily in adult or fully differentiated tissues) can be influenced by extrinsic messages received from the local environment, which provide an external polarity cue.

It is noted that in both current models, the actual asymmetric division is believed to occur after some evidence of asymmetric behavior in the cytoplasm. However, the observed changes in the cytoplasm are likely not the cause of asymmetric division, but rather

steps along the path of asymmetric division determined and initiated by unseen activity in the nucleus. For example, in intrinsically guided asymmetric division, the cytoplasm is seen to polarize (as it might in a chemotactic response, but without a preferred axis of orientation) before the obviously asymmetric division occurs. The point is that the cytoplasm does not 'know" to reorganize itself, the instructions must have come from the nucleus. Thus, the first step in this process must have been some change in the nucleus. Contrary to the notion that the nucleus is reprogrammed after the asymmetric mitosis is complete, the nucleus must be reprogrammed before there is evidences in the cytoplasm that an asymmetric division is about to occur (Figure 1).

A New Hypothesis for Asymmetric Division

The new hypothesis for asymmetric division, presented here, is summarized in Figure 1. During normal expansion of a clone, the original parental strands of DNA are copied during DNA synthesis, but the new strand does not have any of the parental epigenetic markings (i.e., methylcytosine bases in CpG units). The epigenetic imprinting of the parent strand must be added to the new strand during the G2 phase. In normal symmetric division, this process ensures that the daughter cells will not be different from the parent. In the hypothesis of asymmetric division presented here, the G2 phase is the point where the asymmetry is introduced prior to asymmetric division.

The key difference between the new hypothesis and the accepted hypothesis for asymmetric stem cell division during development [4] is that the initiating step is envisioned as induction of new epigenetic imprinting during G2 of a preliminary cell division. I have speculated elsewhere [7, 8] as to what coordinates and initiates the induction of asymmetric imprinting in the stem cell line during development. The actual mechanism for blocking and modifying the epigenetic imprinting was proposed to be DNA and histone methylation guided by noncoding-RNAs [2, 9-15] generated in response to programmed developmental cues [2, 9, 16-18]. After the induction of the asymmetric imprinting in double stranded DNA, it is proposed that the tetraploid cell completes the cell cycle through mitosis. The daughter cells with different imprinting on each strand of their DNA then prepare for another cell cycle. This second cell cycle may include the asymmetric changes in the

cytoplasm that are currently viewed as initiating the intrinsic asymmetric stem cell division. However, these asymmetric changes in the cytoplasm may be incidental to or a result of the asymmetry already established in the nucleus. The notion in the currently accepted model (intrinsic or extrinsic) that the cytoplasm causes reprogramming of the nucleus is poorly supported by experiment.

The important feature of the hypothesis advanced here is that in the cell cycle of the asymmetrically imprinted daughter cells (i.e., the second cell cycle in Figure 1), the G2 phase is normal, i.e., each strand of DNA is used as a template for both the base-pairs and the epigenetic imprinting of the grand-daughter cells. At this point, the tetraploid cells have two distinctly different sets of chromosomes with respect to imprinting. When mitosis occurs, one cell retains the original stem cell imprinting and the other cell carries a new pattern of imprinting; thus, asymmetric stem cell division and differentiation have occurred.

For this process to work, the differential methylation of DNA strands either (i) must only involve one chromosome at a time or (ii) there must be a mechanism for ensuring the segregation of all the reprogrammed chromosomes into the same new cell [19].

Proof of Principle and the Immortal Strand Hypothesis

Proof of the principle embodied in this hypothesis (Figure 1) can be found in studies involving labeling of DNA strands with halogenated-deoxyuridine (halo-dU) [20]. Using this technique, it is observed that the parental DNA strand is retained by the parent (older) stem cell as would be predicted by this model because the parental base-pairs carry the unmodified epigenetic marks [21]. Specifically, you will notice (Figure 1) that all the original strands of DNA are retained in the original stem cell clone (as in the "immortal stand hypothesis" [20]) but no complex mechanisms of chromosome segregation are invoked as was done by Lew et al. [19]. Moreover, as discovered by Fei and Hunter [22-24] this process takes two generations to accomplish.

The immortal strand hypothesis dates to 1975 when Cairns [25, 26] proposed that it would help preserve the integrity of the stem cell genome through many generations of division. A number of people have confirmed that the parental strand is conserved and/or segregates with the older stem cell [27-30], but they have not explained how this happens. It happens because the epigenetic marking

on the original strands are the factors that define the parental stem cell. It is noted that the DNA that retains the original parental imprinting also retains full telomere length and is not "aged" during the process, whereas the newly synthesized strands with new imprinting progressively lose telomere length generation-by-generation. The parental strand also avoids replication errors because it is always the template strand.

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