

Revisit Semi-Conservative DNA Replication and Immortal DNA Strand Hypothesis

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HIGHLIGHT

A paper describing a very fundamental problem in biological research and relating many cutting-edge research findings was submitted to “top” journals but none of them were interested to publish it. Why?

ABSTRACT

“Semi-conservative” DNA replication and “immortal” DNA strand hypothesis are both reexamined in light of an alternative view on cell life. The proposal of one mother cell reproducing one or more daughter cell(s) and the mother cell always keeping its original template DNA strand makes more sense than the conventional “one mother two daughter” concept and challenges the immortality claim for the template DNA strand.

KEY WORDS

DNA replication, Cell reproduction, DNA age, Cell age, Aging, Life span, Death, Immortality

Almost half-century ago Meselson and Stahl demonstrated the semi-conservative nature of DNA replication (1). This wet-lab experiment conclusively proved the correctness of the theoretical double-helix model for DNA structure as proposed by Watson and Crick (2). To a large degree, Meselson and Stahl’s application verification contributed to the eventual awarding of a Nobel Prize in 1962 to the latter two plus Maurice Wilkins (http://nobelprize.org/nobel_prizes/medicine/laureates/1962/index.html).

Theoretically semi-conservative replication would naturally create a genuine age difference between the template and the copied DNA strand. However, the cellular significance of this molecular-level age difference is still largely unexplored. This is because conventional wisdom

has so far insisted on a groundless claim that one mother cell divides into two daughter cells (3). In fact, existing publications demonstrating some innate and intrinsic age distinctions between two cells formed from one cell (4) (5-9) have been totally ignored by the mainstream biologists.

Another main obstacle for advancing cell age difference between two cells formed from one cell is the traditional and still dominant view of random segregation of DNA strands or sister chromosomes in cell division. However, my previous reading on relevant literature convinced me that such conclusion might be invalid (10). In fact, I had found some evidences that are more consistent with a not just non-random but also very regular pattern of DNA strand/chromosome segregation (unpublished paper).

The scattered information of non-random DNA strand distribution/chromosome segregation was taken into consideration when I submitted three proposals (10) in January 1992 intended for my PhD dissertation study. The grand goal of these proposals was to demonstrate some genuine age differences between two cells (using bacteria as examples) formed from one cell and show that even prokaryotic cells have cytoskeleton and thus can carry out some non-random processes.

Unfortunately, none of above three proposals was accepted by my PhD committee because, for one thing, they were too remote from my main study direction – environmental microbiology. Nevertheless, while still working on my obligated research and finishing my PhD dissertation, I also used my spare time to do some preliminary but crucial experiments on the above lines of thinking using limited resource obtained through intentional selection of some graduate courses to gain access to some required equipments.

My “amateur” experiments revealed the true chronological aging in bacteria whether it has an asymmetric body and thus divides asymmetrically or it has a symmetric body and thus divides “symmetrically” (4, 8, 9). These observations led to a generalized microbial/cell life model which claims that the life cycle of cell should include at least three major phases: juvenile, adult, and senescent phase and reproduction is just one part of this life-span in the adult stage (6). This revised view of microbial/cell life actually made it possible to conceptually unite a dichotomous biology under some common life principles (11).

The search for bacterial cytoskeleton was carried out by my wife (a PhD student in a small laboratory studying eukaryotic cytoskeleton) to the extent that we found at least some cytoskeleton-like structures and purified some proteins with similarity to eukaryotic cytoskeletal molecules. The results were presented in a university research conference (12, 13). Unfortunately, our goal of determining the protein sequences and then reverse deciphering their DNA sequences were too ambitious for a PhD-level research at that time and too expensive for a small lab lacking a direct grant support. So we had to stop that research half way.

As to my third proposal, I was really unable to carry it out under an amateur status because its experiment requires using some radioactive materials (the only choice at that time for labeling DNA). The strategy was to selectively label one strand of DNA and then follow its segregation pattern over generations under conditions that each individual cell can be reliably tracked. I believed that such experiment would provide very

convincing arguments to support my view that, after cell reproduction, one is still the old mother but the other is a true daughter.

It turned out that my career path has not yet provided me a luxury to complete my long-wanted experiment in a wet lab. However, I have mentally performed such experiment many times and felt that the results of my *in silico* experiments are very correct as I saw more and more accommodating evidence appeared in literature. So I thought that it might be a right time to publish my “dry-run” results as a hypothesis so that scientific advancement in this area would not be further delayed.

In February 2005 I submitted to *Nature* a letter which was formulated in the format of the famous Watson and Crick DNA double helix paper. However, *Nature* found my ideas “do not seem to be of sufficiently broad interest or topicality to our general readership” and rejected it right away. I found this decision ridiculous since right at that time a paper describing bacterial aging and death was published in a very high-profile fashion in a newly emerged top journal (14) and was even heralded world-wide by other traditional top journals and popular media as a major breakthrough (in fact it was only an imperfect repetition of my early findings (15)). So I complained to the Editor-in-Chief of *Nature* and even attached some very popular reports on the so-called “new discovery” (in fact a true credit robbery (16)). Amazingly, *Nature* still insisted on its early decision and even stated that “it (my hypothesis) is unlikely to have an immediate impact on the thinking of other researchers”. This letter was later published in *Logical Biology* (17). A more comprehensive paper describing the aging and differentiation framework in multicellular organisms was also published there (18).

I should emphasize, my hypothesis not only depicted a precise connection between DNA aging and cell aging but also clearly showed the respective contribution of genetic (DNA base sequence) and epigenetic (DNA base modification) information to the formation of the biological characters of each individual cells or unicellular organisms. The extension of this DNA-cell aging axis into the framework of understanding development of multicellular organisms sheds many new insights to better understanding of some key biological processes such as cell differentiation and the stemness of stem cells.

Thus *Nature*'s judgment on the potential impact of my hypothesis is deadly wrong. Without doing any in-depth search and just glancing over the table of contents of some recent issues of some very top

journals including *Nature* it is almost impossible to avoid reading papers that report asymmetric cell division and even non-random DNA segregation (a few examples are referenced here (19-21)). Interestingly, none of the authors in these later publications have cited my earlier publications on the very same subject, despite the fact that my earlier publications had already presented much clearer evidence and more detailed descriptions on the very likely mechanisms for the phenomena they later found as “striking”.

Ironically, while I can hardly avoid seeing accommodative evidences being published by top scientific journals, my correspondences to top journals pointing out this verification of my old discovery or hypothesis if someone insists were repeated rejected. More surprising to me is that almost every new report on finding the cosegregation of template DNA strands in asymmetric cell division has attached its observations to the “immortal DNA strand” hypothesis advanced by John Cairns (22). However, in my opinion, the “immortality” claim for the DNA strand is logically fallacious and scientifically wrong.

The core component of Cairns’ “immortal strand” hypothesis is the claimed process of passing the template DNA strand only to the “immortal daughter cell” and always distributing the copy DNA strand to the so-called “mortal daughter cell”. Based on this assumption, it was further reasoned that the “immortal strand of DNA” can serve the purpose of protecting DNA against errors of duplication and thus help the survival of stem cells against the natural selection of fitter mutated cells (22).

However, there are some obvious logical and scientific problems with this core component of the “immortal strand DNA” hypothesis.

First, it is logically wrong to use term “immortal” and “mortal” for describing some thing that is actually unrelated with the mortality of the DNA or the cell. After carefully reading Cairns’ paper, I realized that what he should really state is that, since the template strand DNA is always passed to the stem cell (in his view) and the copied strand of DNA is always passed to the non-stem cell (also in his view), stem cell has the great potential of avoiding DNA replication-related mutation since which also requires homozygosity to get the mutation on both strands. Therefore stem cells also have no or less chance for developing cancer. However, even if this reduced chance of oncogenesis holds up true for the stem cells, it does not prove that stem cells are “immortal”. Simply

speaking, original template DNA strand should not be directly translated into “immortal DNA strand”.

Secondly, it is scientifically wrong to claim that an old DNA strand (formed at least one generation earlier) is capable of being “immortal” while a young DNA strand (have to be formed at a next replication/copying process) must be “mortal”. If people doubt this scientific reasoning, please show under what physical/chemical principle that old object/material would or should live forever but the same young object/material has to die?

Thirdly, Cairns argued that DNA in some stem cells may be “permanently labeled” and thus that DNA strand must be “immortal”. However, long-term labeling of a molecule may not necessarily correlate with infinite living/function of that molecule. In fact, the permanent status of the labeling is only discussed in some relative term as to the observation period which does not necessarily cover the complete life-span of the cell or the organism.

Therefore, while Cairns’s hypothesis correctly recognized the fact that one of the paired cells formed from stem cell can be different from the other and thus the stem cell division is asymmetric even if the cells formed from this division are apparently indistinguishable, Cairns failed to recognize the fact that the distinctions between any paired cells formed from one cell are actually some reflections of their truly generation- and age-differences.

Cairns believed that primer mutation in stem cells occurs only at the time when these cells are created or “regenerated”. This statement is only half-true in the sense that the so-called “regeneration” of stem cells in fact does not occur. The apparent “regeneration” or the so-called “renewal” of the stem cell is in fact a total misunderstanding of cell life. The “renewed” “daughter” stem cell is actually the original mother stem cell that just lived through its one round of reproduction!

Cairns reasoned that the fate of the two “daughters” of any “immortal” stem cell is determined by their position in space. This external influence on the so-called “cell fate” is undeniable but ruling out any intrinsic control on the “cell fate” is unjustified. In my opinion a major factor determining the so-called “fate” differences between any cells formed from one cell is its internal genome status which is largely reflected by the epigenetic modifications rather than the genetic mutations. In a cell lineage the degree of “stemness” and the timing of the expression of these different levels of “stemness” may be a result of the devolution of the evolution-integrated

genome and is related with the aging process of the cell.

Cairns emphasized that asymmetric cell division may occur only in a few stem cells. However, this statement may be narrow-minded and I do not believe that the selective template DNA strand segregation occurs only in stem cells or only at the “stemness” stage of the cells. I have hypothesized that this selective segregation of age-different DNA strands into respective age-different cells happens normally in the reproduction of all types of cells (4-9, 17, 18, 23-31). However, some prior knowledge on the cell reproduction cycle length and the inter-reproduction cycle time has to be obtained before a right scheme of labeling and chasing experiment can be found to reveal this regularity in DNA strand segregation.

In my view, the oldest template strand of DNA is in fact also the most “senescent” strand of the DNA (17, 18). It is thus also the DNA strand that most likely accumulate more aging-related damages and thus become the most vulnerable strand of DNA (32). In this sense, these DNA strands are not only not immortal but also become the Achilles heel for the cell mortality.

The template DNA strand is not immune to DNA replication error at all because, despite its infrequent use in the mother cells, it nevertheless has to be used some times in the life-span of the mother cell when it does need to reproduce. Since this long-lived template DNA theoretically has greater chance to be damaged over the course of living it likely contains more defects than other later copied strand of DNA. This genetically mutated/base sequence altered or epigenetically changed/base modification altered template DNA strand may be the original source of mutation or epi-mutation. Furthermore, such fragile old DNA strand may even have greater tendency to malfunction during the DNA replication process. Thus, it is unlikely for the template DNA to be some “immortal” form of DNA that can provide protection against natural selection. To the contrary, it may be a source of errors that can be copied into its offspring and thus allow them to become cancer cells even if these errors just stay “harmlessly” in the “resting” (non-reproductive) stem cells.

In fact, Cairns’ claim of reduced mutation in template “immortal” strand of DNA has not being validated by experimental evidence. His claim of “immortal” strand protection of cancer in stem cells is also contradictory with the increasing findings of stem cell cancers (33, 34). Thus, in light of all literature evidences and my above arguments, people should realize the danger of attaching solid

new findings to an already moribund if not totally mortal hypothesis.

Finally, to help readers better understand my view and how it could better explain the phenomena observed by Meselson and Stahl (1), I wish to add my annotations to the classical drawing of Meselson and Stahl to reflect the distribution of N¹⁵-label in the DNA strands in a cell lineage (Fig. 1). I also wish to re-publish a drawing (Fig. 2) of the distribution of age-different DNA strands upon cell reproduction to emphasize the epigenetic influence on the cell difference (17, 18).

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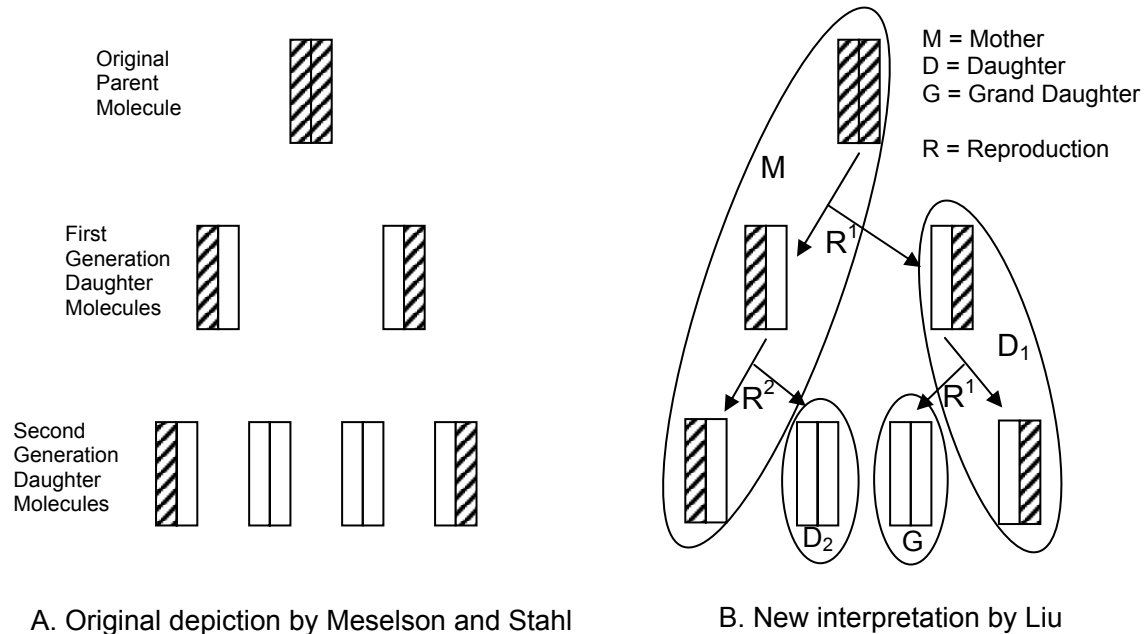


Figure 1. New insight on an old observation.

Upon semi-conservative DNA replication of N^{15} -labeled DNA strands (both strands were labeled) mother cell use N^{14} in the chasing medium to build new copy of DNA and thus its retained DNA will become half- N^{15} -labeled. Its first daughter will also be half- N^{15} -labeled since it inherits one N^{15} -labeled DNA strand. The mother cell will always keep its original template N^{15} -labeled DNA strand and thus always half- N^{15} -labeled. However, its later reproduction(s) in the N^{14} chasing medium will always produce daughter(s) with no N^{15} -label. The first daughter will also always keep its inherited N^{15} -labeled old DNA as template and its daughter (grand daughter of the original mother) will all be N^{14} -labeled. As the population grow and the number of N^{14} -labeled cells will keep increase. Please note the key emphasis of placing the same cell in different ages/times of reproduction in one cell-shaped circle to show the family composition in different times. Subscripts and superscripts indicated siblings of the same generation and sequential reproduction events by the same mother, respectively.

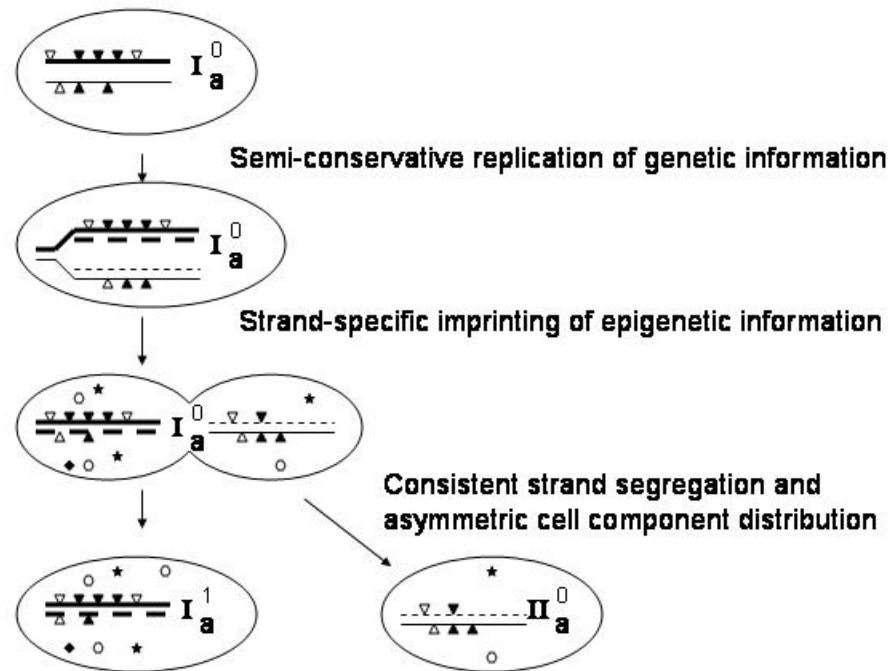


Figure 2. A hypothetical model for linking DNA age with cell age and for highlighting contributions from both genetic and epigenetic components to the intrinsically asymmetric cell reproduction (Originally published in *Logical Biol.* 5: 51-55, 2005).

Roman numbers represent cells of different generation and age (or cell types in a differentiation sense). The superscript number represents reproduction event experienced by the cell. The subscript letter identifies each individual offspring reproduced from the same parent cell. Old and new original DNA strands are indicated by the solid thick and the solid thin lines, respectively. Their replicated complementary strands are indicated by the dashed thick and dashed thin lines, respectively. Filled and hollow triangles on DNA strands stand for epigenetic imprinting on DNA of various types and numbers. Objects of various shapes in the cellular space represent other components that are differentially distributed in cell reproduction.

* This paper was submitted to *Nature* on 2006-08-15 (without the figures) and was rejected on 2006-08-24. Then it was submitted to *Cell* on 2006-08-24 and was rejected on 2006-08-25. Finally it was submitted to *Science* on 2006-08-27 and was rejected on 2006-09-12. The publication here is as exactly as it was finally submitted to *Science* except for the added highlight and keywords.

PS: Cover letters submitted along with the manuscript and the rejection emails received

To *Nature*

Dear Dr. Campbell and *Nature* editors,

Early last year *Nature* rejected my Hypothesis paper entitled “**Linking DNA Aging with Cell Aging and Combining Genetics with Epigenetics**” on the ground of its lacking of general interest because “it is unlikely to have an immediate impact on the thinking of other researchers”.

Now I am submitting another Essay-type paper entitled “**Revisit semi-conservative DNA replication and immortal DNA strand hypothesis**”. This paper critically evaluated the cellular consequence of semi-conservative DNA replication and the logical fallacies and scientific mistakes of the “immortal DNA strand” hypothesis that was originally published in *Nature* three decades ago and recently highlighted again in *Nature*.

Looking back I wish that you, *Nature* editors, and *Nature* reviewers would realize how big a mistake that *Nature* has made by repeatedly rejecting my logical and valid observations and hypotheses. The damage of a fifteen-years-long rejection to some truly ground-breaking discoveries may be insignificant to a true and dedicated scientist but cannot be underestimated to society as a whole.

With all the emerging new evidences indirectly accommodating if not directly supporting my previous observations and hypotheses (published anyway despite the “low impact” status of the journals they reside) I wish that *Nature* will regard my new submission as not only insightful but also forward thinking.

Thank you for your attention. I am looking forward to hearing from your earlier decision.

Shi V. Liu

To *Cell*

Dear Editor,

I am submitting an Essay entitled “**Revisit Semi-Conservative DNA Replication and Immortal DNA Strand Hypothesis**” to be published in the Leading Edge section of *Cell*.

This Essay expresses my opinions in a concise manner on some most important biological topics. It provides truly fresh insights on to some enigmatic problems. It has a live historical perspective, discusses some landmark papers in biology, and highlight what we can learn from the dark suppression of some ground-breaking discoveries. It certainly fits *Cell* Essays’ feature of containing “more speculation than traditional formats”.

The length of the body text is 2486 words. It contains 34 references (due to its coverage of a wide-scope topic and the rich history of some still unrecognized publications). Two figures are included.

I believe this Essay would be a piece of publication that is hard to find in a long interval of scientific research. It certainly touches the heart and soul of cell research.

I hope that *Cell* will find this essay very useful and suitable for its broad readership. I also welcome editor’s constructive suggestions to further improve this essay to satisfy the high standard of *Cell*.

Thank you for your attention!

Sincerely,

Shi V. Liu

Reply by *Nature*

Dear Dr Liu

Thank you for sending us your article "Revisit semi-conservative DNA replication and immortal DNA strand hypothesis", which we have considered for our Commentary section, but which I regret we are unable to publish. As I'm sure you are aware, pressure on our limited space is severe, so we can offer to publish only a few of the many submissions we receive. Commentary pieces in *Nature* are usually journalistic articles, rather than the sort of technical analysis you propose. Ultimately, therefore, we suspect that the specific arguments you provide would be better suited to publication, and discussion, in the specialist literature. Once again, I am sorry I could not provide a more positive response on this occasion, but I wish you luck publishing your article elsewhere.

Reply by *Cell*

Dear Dr. Liu,

Thank you for your presubmission enquiry regarding an Essay for *Cell*. Unfortunately we are unable to offer to publish your Essay. Most Leading Edge articles are commissioned and there is fierce competition among unsolicited pieces for our very limited space. We realize that you have submitted a number of articles to *Cell*'s Leading Edge section over the past few months and that we have been unable to publish any of them. Mindful of the time that you must spend on writing and submitting these pieces, we would like to suggest that you should consider submitting your articles to a more specialized journal in the future.

Reply by *Science*

Dear Dr. Liu:

Thank you for submitting your manuscript "Revisit Semi-Conservative DNA Replication and Immortal DNA Strand Hypothesis" for consideration as a Perspective. Although your analysis is interesting, we feel that the scope and focus of your paper make it more appropriate for a more specialized journal. We are therefore notifying you so that you can seek publication elsewhere.

We now receive many more interesting proposals for Perspectives than we can publish, and right now, our pipeline is quite full. Most of the Perspectives that we publish are associated with papers in *Science*. That leaves very limited space for commentaries on other topics and as a result, we have to turn many away. Therefore, our decision is not necessarily a reflection of the quality of your commentary but rather of our stringent space limitations.

We wish you every success when you submit the paper elsewhere.