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Cellular replication limits in the Luria–Delbrück mutation model

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HIGHLIGHTS

- First Luria–Delbrück model that takes into account cellular replication limits.
- Model the emergence of mutants that escape replicative senescence.
- Results on the mean, variance, distribution, and asymptotic behavior of the mutant population.
- Discuss applications, including telomere crisis and fluctuation analysis.

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ABSTRACT

Originally developed to elucidate the mechanisms of natural selection in bacteria, the Luria–Delbrück model assumed that cells are intrinsically capable of dividing an unlimited number of times. This assumption however, is not true for human somatic cells which undergo replicative senescence. Replicative senescence is thought to act as a mechanism to protect against cancer and the escape from it is a rate-limiting step in cancer progression. Here we introduce a Luria–Delbrück model that explicitly takes into account cellular replication limits in the wild type cell population and models the emergence of mutants that escape replicative senescence. We present results on the mean, variance, distribution, and asymptotic behavior of the mutant population in terms of three classical formulations of the problem. More broadly the paper introduces the concept of incorporating replicative limits as part of the Luria–Delbrück mutational framework. Guidelines to extend the theory to include other types of mutations and possible applications to the modeling of telomere crisis and fluctuation analysis are also discussed.

1. Introduction

The Luria–Delbrück experiment investigated whether mutations in bacteria arise spontaneously or as an adaptive response [1]. The answer led to a rich mathematical theory, with important applications in the calculation of mutation rates [2], the emergence of antibiotic-resistant microbes [3], the study of drug therapy-resistant cancer cells [4,5] and cancer genetics [6,7]. Theoretical advances include the analysis of the probability distributions [8,9], asymptotic properties [10], numerical methods for fluctuation analysis [11], and the accuracy of estimates for the mutation rates [12]. Extensions of the theory include different cell cycle distributions and growth laws of wild type cells [13,14]. For a review and more recent advances see [15,16].

Currently an underlying assumption of the theory is that cells are capable of an unlimited number of divisions. This assumption is appropriate to model mutations in bacteria, it does not apply however, to the majority of cells in the human body. Normal human somatic cells are capable of a limited number of divisions, a phenomenon known as replicative senescence or Hayflick's limit [17]. Hence, when we consider the somatic evolution of human cells, it is fundamental to understand how replicative limits affect the emergence, dynamics, and distribution of mutant populations. Here, we present the first attempt to address explicitly the role of replicative limits in the Luria–Delbrück mutational framework.

Replicative senescence is linked to the shortening of telomeres during cell division, which are repetitive sequences of DNA found at the end of linear chromosomes [18]. Replication limits protect against cancer by limiting the size of a clonal cell population and by reducing the possible number of cells divisions, when mutations typically occur. Cells can escape replicative senescence by expressing telomerase, an enzyme that extends telomere length [19].

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Table 1
Summary of the different formulations discussed in the paper.

	Luria–Delbrük	Lea–Coulson	Bartlett
Growth of wild type cells	Deterministic	Deterministic	Stochastic
Mutations	Random	Random	Random
Growth of mutants	Deterministic	Stochastic	Stochastic
Notes	Only knowledge of the average behavior of mutant clones is needed.	Captures additional stochasticity in the mutant population. Requires knowledge of the p.g.f. of mutant clones.	Fully stochastic version. Potentially the most realistic. Not a filtered Poisson process. Hardest to analyze.

Essentially all human cancers acquire mechanisms to maintain telomere length, most often through high levels of telomerase expression (90%) [19], and less frequently through the alternative telomere lengthening pathway (ALT) (10%) [20]. The stage of tumor development at which cells start expressing telomerase is probably cancer-type-specific. Proliferation in telomerase negative cells after the inactivation of cell-cycle checkpoint pathways can lead to crisis, a phase characterized by widespread cell death and genome instability. Cells can emerge from crisis immortalized through telomerase activation [18]. This sequence of events might play an important role in breast cancer [21]. In other cancer types telomerase expression might occur at earlier stages [18]. If cancer originates in a telomerase positive stem cell, full progression towards malignancy might involve instead the up-regulation of telomerase activity. The finding in tumors of cells with stem cell characteristics has led to the concept of cancer stem cells (CSC). There is debate over the cell of origin of CSCs, whether they originate from normal stem cells or from differentiated cell types, which acquired stem cell characteristics. In multiple types of cancer there is evidence that the initiating mutations originate in cells with limited proliferative potential, such as progenitors (for a review see [22]).

Here, we consider mutations that allow cells to bypass replicative limits, and develop the framework in terms of three formulations with different levels of randomness (Table 1). The manuscript is organized as follows. First, we consider the Luria–Delbrück formulation, which assumes that mutant and wild type cells grow deterministically. This formulation is useful when only the average behavior of mutant clones is known, as occurs in two of the applications considered in the Discussion section. In the article we focus on the case where mutant cells grow exponentially and derive results for the mean, variance, and asymptotic behavior. In this context (exponential mutant growth), the results for the Luria–Delbrück formulation are then used to derive statistics for the Lea–Coulson model, which assumes that wild type cells grow deterministically and the mutant population grows stochastically. In principle the Lea–Coulson formulation is preferable, because it captures additional stochasticity in the mutant population; however, the probability generating function of the mutant clones is often not known. Despite this limitation, the applications for the Lea–Coulson formulation go beyond the single mutant with exponential growth case. In particular, we discuss a possible application to telomere crisis. Next, we discuss Bartlett's formulation, which assumes stochasticity at every layer of the process. In this section, we derive the stochastic master equation for the entire cell population, which should form the basis for future research into the fully stochastic version of the problem. We also investigate the probability of escaping replicative limits. Understanding this quantity is crucial to evaluate the effectiveness of replicative senescence as a tumor suppressor pathway. Throughout the paper we emphasize the comparison between the different statistics when replication limits are included with those from the classical model, where they are not. We end by discussing applications of fluctuation analysis to estimate the rate of telomerase activation.

2. Cellular replication limits

To model replicative limits we assume that each cell has a replication capacity $\rho \geq 0$. When a cell with replication capacity $\rho > 0$ divides, it produces two daughter cells with replication capacities $\rho - 1$. Cells with replication capacity $\rho = 0$ become senescent and stop dividing (Fig. 1(A)).

Let the rates of cell division and death be a_{div} and a_{die}. If we denote the normalized division rate by $q = a_{\text{div}}/(a_{\text{die}} + a_{\text{div}})$, then $a_{\text{div}} = q(a_{\text{die}} + a_{\text{div}})$ and $a_{\text{die}} = (1-q)(a_{\text{die}} + a_{\text{div}})$. Notice that in general $0 \leq q \leq 1$ and if the cell population initially grows $q > 0.5$. We can then express the model in terms of dimensionless units of time by making $a_{\text{die}} + a_{\text{div}} = 1$. If $x_\rho(t)$ is the number of cells with replication capacity ρ at time t and k is the maximum replication capacity, then the time evolution of the cell population is described by the system:

$$\begin{cases} \dot{x}_k = -x_k \\ \dot{x}_{k-1} = 2q\,x_k - x_{k-1} \\ \dot{x}_{k-2} = 2q\,x_{k-1} - x_{k-2} \\ \quad\vdots \\ \dot{x}_0 = 2q\,x_1 - (1-q)\,x_0. \end{cases} \tag{1}$$

We refer to propositions in the Supplementary material (see Appendix A) with the letter "S" followed by a roman numeral. If $X_{\text{tot}}(t;q) = \sum_{\rho=0}^{k} x_\rho(t)$ and there is a single cell with replication capacity k at $t = 0$, from *S1* we have:

$$X_{\text{tot}}(t;q) = e^{-t}\left[2^k \sum_{n=k}^{\infty} \frac{(qt)^n}{n!} + \sum_{n=0}^{k-1} \frac{(2qt)^n}{n!}\right]. \tag{2}$$

Fig. 1(B), plots the trajectory of $X_{\text{tot}}(t;q)$ for different values of k. If $0.5 < q < 1$, the cell population first grows on account of the positive net growth rate ($a_{\text{div}} > a_{\text{die}}$). However, as time progresses more cells hit Hayflick's limit and stop dividing. When this occurs the cell population starts to decrease as most cells become senescent and eventually die. For an analysis of the stochastic version of (1) see [23].

Here we focus on mutations that activate telomerase. Telomerase allows cells to bypass replicative limits, but it does not reverse senescence [24]. For this reason a telomerase-activating mutation that originates in a senescent cell is a dead end. Thus, from here on we focus our attention on the dividing (non-senescent) fraction of the cell population $X(t;q) = \sum_{j>0} x_j(t)$. In terms of the incomplete upper gamma function $\Gamma(k,t) = \int_t^\infty s^{k-1}e^{-s}\mathrm{d}s$, we find:

$$X(t;q) = e^{(2q-1)t}\,\Gamma(k,2qt)/\Gamma(k). \tag{3}$$

Eq. (3) can be modified to include different initial conditions. If k is the maximum replication capacity found in the population at time $t = 0$, then the total number of dividing cells equals $e^{(2q-1)t}\sum_{j=1}^{k} x_j(0)\Gamma(j,2qt)/\Gamma(j)$. From here on we focus on populations arising from a single founding cell (Eq. (3)). Results for different initial conditions can be derived from the fact that the processes arising from each of the subpopulations $x_j(0)$ are independent of each other.