# Bridging the Generation Gap: Tracking Movement of Functional Proteins Across Generations in *Escherichia coli*

Richard Dennis '17 and Dr. Joan L. Slonczewski
Department of Biology, Kenyon College, Summer Science 2014

## Abstract

Aging has long been thought of as a fate relegated to multicellular organisms, while "simpler" single celled organisms evaded such a fate at the expense of dying when resources became lacking, or they encountered some other stroke of bad luck. Yet studies show that even under ideal conditions, such cells die out, and there is evidence of the possibility that uneven ages of poles in individual cells of *Escherichia coli,* otherwise symmetrical and allegedly identical in division, may be responsible for varying levels of fitness in daughter cells; those containing primarily younger poles tending to reproduce at faster rates compared to those containing older poles (4). We examined strains of *E. coli* with housekeeper proteins fused to Green Fluorescent Protein (GFP) to determine whether they moved in particular ways as the cells divided. All GFP observed was present in the cell prior to the beginning of observation, so it was known that any fluorescence observed was based on proteins that had been formed before the experiment began. A loss of cellular fitness can be relegated to protein damage over time, and older proteins can thereby be attributed to a lesser level of cellular fitness. This experiment attempts to track aging proteins being utilized by *E. coli* cells as they divide to confirm whether or not the cells differentiate between proteins as they age, and whether or not this could be a mechanism involved in the aging process.

## Introduction

***Escherichia coli* appears to divide and age symmetrically. However, this would imply that the cells in a colony do not age, as every daughter cell would effectively in such an instance be the same age as the progenitor of a colony.**

- One Study (2) determined that *E. coli* cells independently segregate proteins to poles, with a greater likelihood of moving undesirable proteins toward older poles.
- Other studies in the Slonczewski lab indicate that *E. coli* cells which contain older poles do tend toward slower rates of division compared to cells containing more newly formed poles.

**Housekeeping proteins are present under normal cellular conditions, and may undergo similar protein segregation processes over time in dividing cells, which could point toward a mechanism behind why aging takes place.**

- By inducing production of a GFP-housekeeping protein fusion in *E. coli*, we can visualize the movement of these housekeeping proteins in the cells.
- Because no more GFP-housekeeping fusion proteins are produced during the experiment, any areas exhibiting fluorescence contain proteins of a known age; by comparing the redistribution of fluorescence over time, we can effectively track the movement of these proteins as they age and the cells divide.

## Methods

**Strain Preparation:** Studied strains were procured from the ASKA collection containing plasmids for induction of GFP-fused proteins of *metG, mdh, purA, cysG, gyrB, recA, fumC, icd,* and *adk* via Isopropyl β-D-1 thiogalactopyranoside (IPTG) exposure, with chloramphenicol resistance cassettes (1).

**Overnight Culture:** Strains were incubated overnight in LBK buffered with 100mM 3-(N-morpholino) propanesulfonic acid (MOPS) at pH 7.0 for standard lab conditions for approximately ten hours, before being removed for inoculation with IPTG at a concentration of .1mM. Cultures were then incubated for a further two hours to allow for some induction of GFP-fused proteins; overexposure to IPTG was seen to heavily inhibit rate of division.

**Microscopy:** Following IPTG incubation, cellular suspensions were spread onto a 40mm coverslip using .35% agarose. Observation was performed using an Olympus BX61WIF-5 microscope. Growth medium lacking IPTG was perfused through the chamber as described in (3). Perfusion media lacked IPTG as compared to the modified overnights in order to ensure that any observed fluorescence in cells was present prior to the beginning of the experiment. Fluorescence was observed via Metamorph Basic software. Single cells were tracked out as they divided, with brightfield and fluorescent images taken at intervals.

## Results

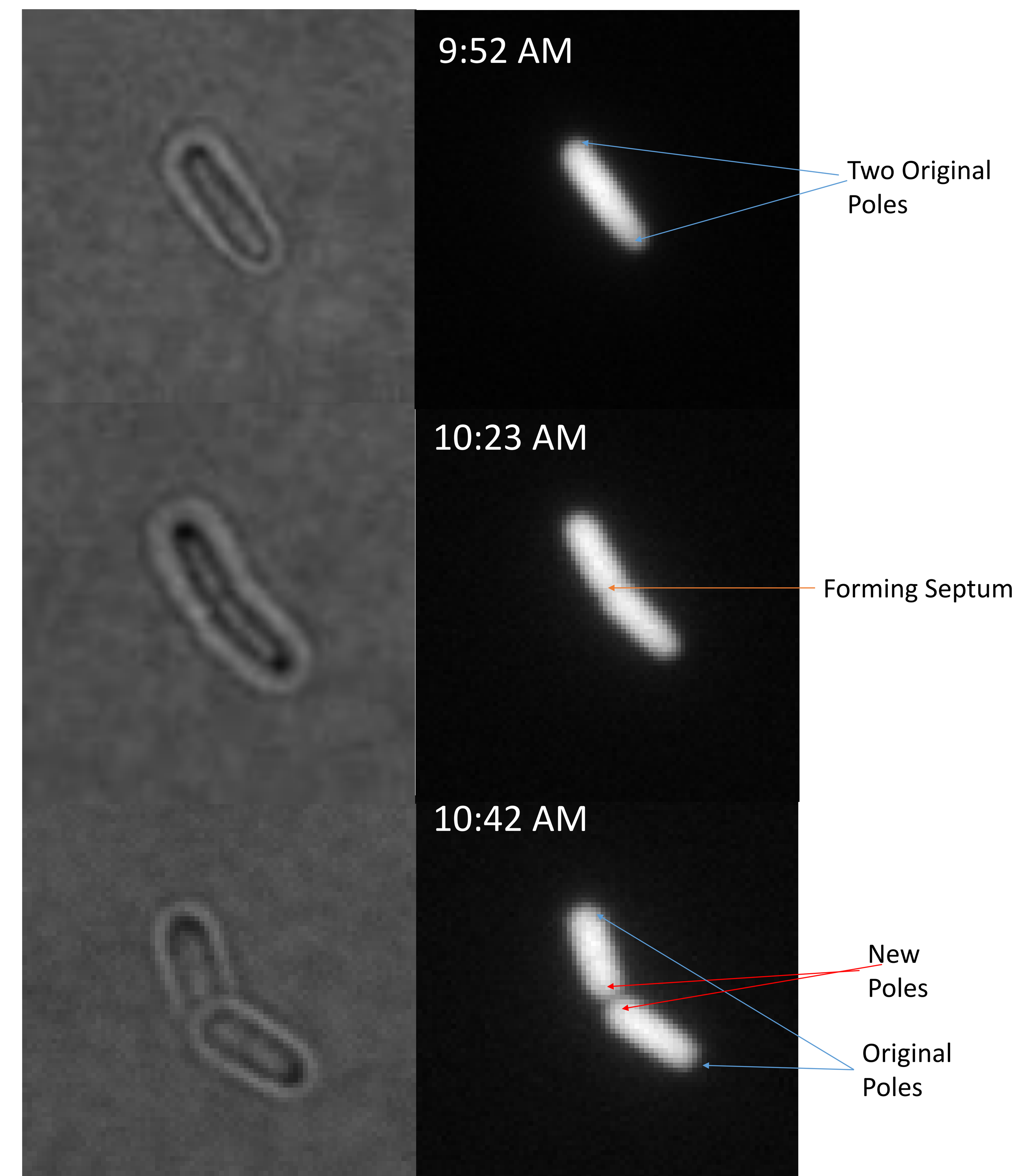


**Figure 1.** (Above) Brightfield (left) and fluorescent (right) images of dividing *E. coli* from strain JW1122, gene *icd*, over the course of about fifty minutes.

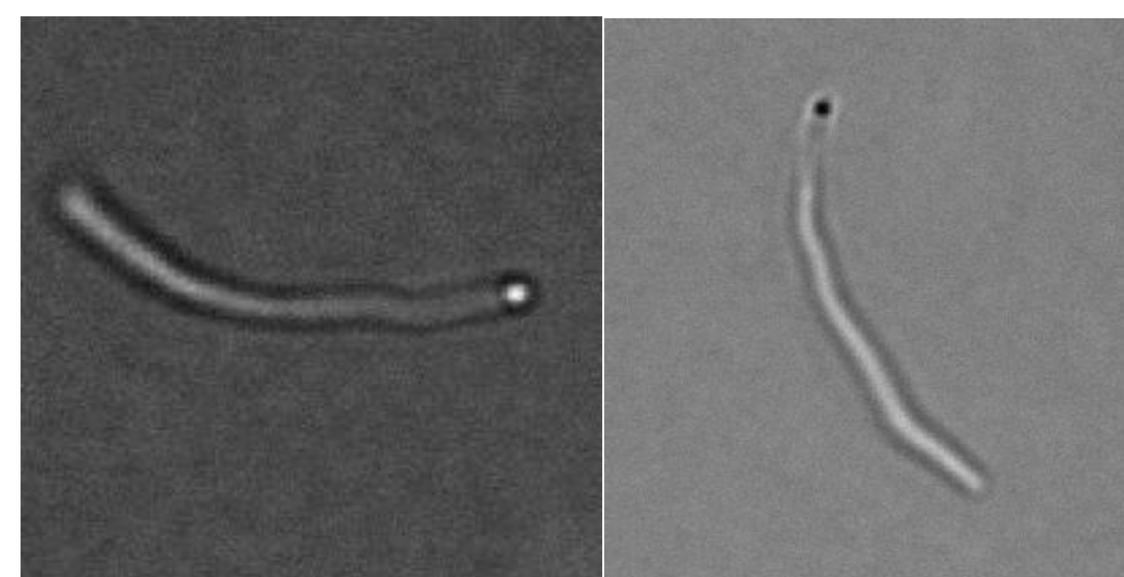

**Figure 2.** Two different cells from strain JW4135, gene *purA*. Extended exposure to IPTG resulted in filamentous growth and inhibited rates of division. Although other literature (5) seems to indicate that the ASKA strains grow normally in lower concentration IPTG regardless of exposure time, such filamentous cells were observed in .01mM IPTG, ten times *less* than the recommended amount for induction of the GFP-fused proteins.

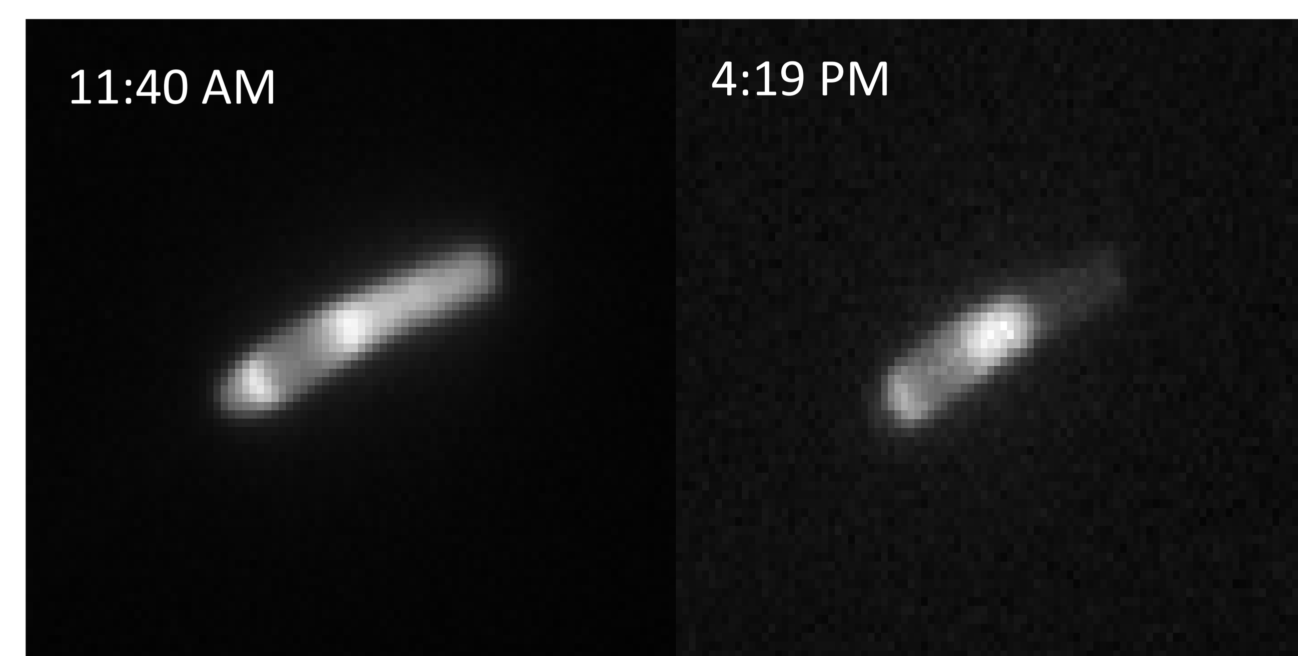


**Figure 3.** The same cell, from strain JW0463, or gene *adk,* at 11:40 AM (left) and at 4:19 PM (right). Although it evidently did not divide, it does show movement of the fluorescent proteins and some degree of partitioning of proteins, in spite of the lack of division.

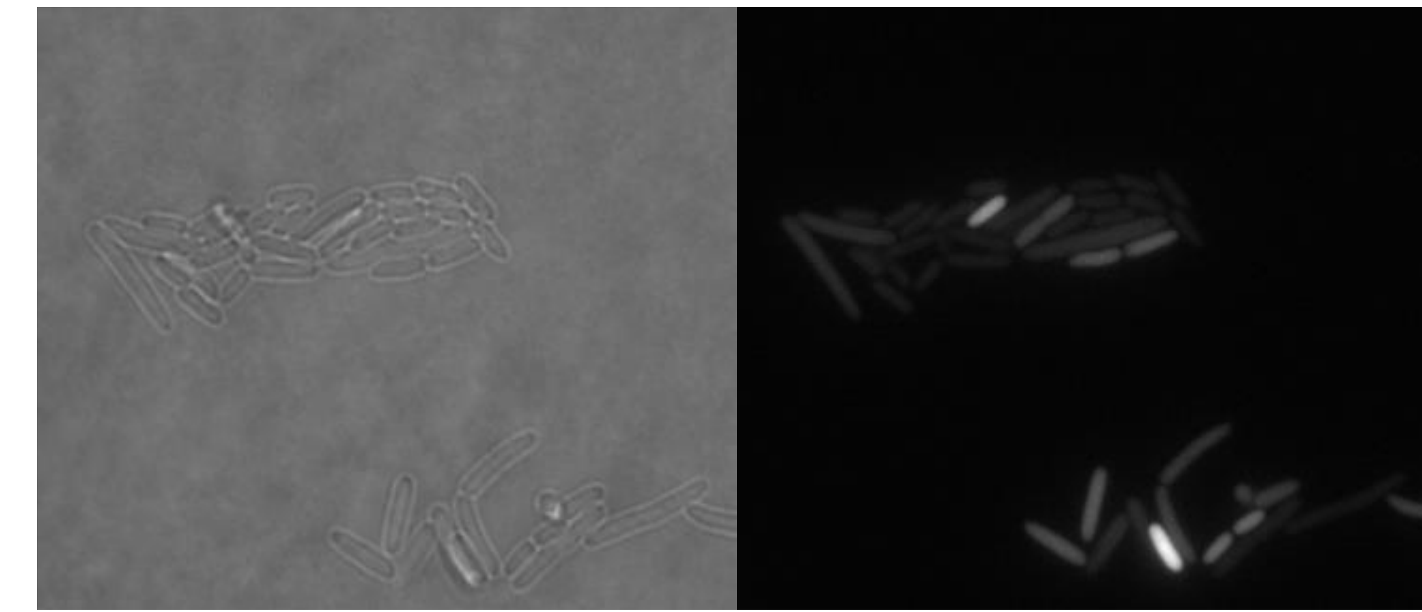

**Figure 4.** Brightfield and fluorescence images of colonies of strain JW0463, expressing GFP-fusion protein *adk*. Though colonies were not watched as they divided out, the discrepancies in fluorescence levels are evident in both colonies, pointing to the potential for segregation of the fluorescent proteins as the cells divide.

## Discussion

- It was found that extended exposure to IPTG was toxic to the *E. coli* cells, resulting in a long, filamentous growth pattern, and interrupting normal division (Fig. 2), consistent with the findings of other literature utilizing ASKA collection *E. coli,* presumably from the additive stress of overproducing proteins.
- The low concentration over a high exposure time resulting in such detrimental effects on the bacteria implies that IPTG could be naturally toxic to the *E. coli* cells; the detectable fluorescence produced by the bacteria at this level of IPTG exposure is on par with what would be produced as background fluorescence regardless. Overproduction of GFP-fused proteins may not be the problem resulting in such abnormal growth patterns. This is what led to the stipulation in the protocol of adding IPTG only two hours before beginning experimental processes.
- Although there is potential evidence in favor of the claim that partitioning of proteins occurs within individual cells as time elapses (Fig. 3) as well as between cells in colonial growth (Fig. 4), lack of proper observation of the occurrence of this partitioning in colonies prevents proper analysis of such results.
- Moving forward, research will continue in hopes of obtaining and replicating data in support of or refuting what is suggested by preliminary results. In addition, research is beginning on strain gyrB, which exhibits a heavily polarized phenotype of GFP-fused proteins as opposed to the more even distribution exhibited in strains such as icd (Fig. 1).

## Acknowledgments

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