



A puzzling pattern of cell-cycle-duration inheritance: just an artefact of changing growth conditions in vitro?

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ABSTRACT

Correspondence re: Sandler, O., S. P. Mizrahi, N. Weiss, O. Agam, I. Simon, and N. Q. Balaban. 2015. "Lineage correlations of single cell division time as a probe of cell-cycle dynamics." *Nature* no. 519 (7544):468-71. doi: 10.1038/nature14318.

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In a recent letter published in *Nature*, Sandler and colleagues presented exciting data on cell-cycle-duration inheritance in a malignant mouse lymphoblast cell line (Sandler et al. 2015). They found that cell-cycle duration, despite a significant correlation within one generation of sister or cousin cells, is lost between mother–daughter cell generations. The authors wondered how cell-cycle-duration inheritance can be lost within one generation (mother–daughter inequality), but can still be present in the next generation (between sisters or cousins). Based on the 'surprising' data, they built a deterministic nonlinear toy model, implying an oscillating internal factor, which determines cell-cycle duration of every new generation of cells in a lineage (Sandler et al. 2015).

To exclude the possibility that the unexpected correlations between sister or cousin cells were not due to the local accumulation of cellular by-products, the cells were cultured in multilayer devices with constantly circulating medium (Sandler et al. 2015). The authors also tested for spatial proximity, but did not find significantly elevated correlations of cell-cycle durations between neighbouring cells. Therefore, it was concluded that growth conditions are stable during several days of long-term time-lapse microscopy (Sandler et al. 2015).

In a complex multicellular organism, it is of great importance that all cells exchange information and act in a collective manner to maintain the health of the individual. This is accomplished through intercellular communication by cytokines, growth factors or other cell signaling molecules. To simulate the physiological conditions, Sandler and colleagues added 10% foetal calf serum to their synthetic medium and supplemented it with a supernatant of exponentially growing cells (Sandler et al. 2015). However, it is widely known that such a cocktail of cytokines, growth factors and all kinds of nutrients and cellular by-products is not stable over a longer time period. Clearly, if growth conditions of mammalian cell culture were stable in vitro, investigators would not routinely exchange the medium every second day or so. Circulating medium is no substitute for adding fresh medium, because it was constantly heated to 37 °C over several days during the whole experiment, which might have even accelerated the normal ageing effect. (Surprisingly, the authors do not mention the temperature of the microscope incubator chamber in the Methods section.)

If my concerns are correct, the changing growth conditions during the experiment resulted in an artefact with the observed pseudo-deterministic pattern. Accordingly, the missing mother–daughter

cell-cycle correlation can be explained by the fact that the mother no longer exists when the daughters are going to divide. With no overlap in time, the changing activity of cytokines and growth factors in the circulating medium automatically alters cell-cycle characteristics between two generations of a lineage. Whereas, related cells of the same generation (sisters or cousins), due to an overlap of more than 90% in time (Sandler et al. 2015), experience identical growth conditions and, therefore, perfectly correlated cell-cycle durations. During a personal communication with one of the authors, I stumbled upon some evidence of changing growth conditions, which I initially overlooked.

[Extended data figure 1](#) shows the mean cell-cycle durations during long-term time-lapse microscopy and although the authors claimed that growth conditions were stable, mean cell-cycle durations obviously varied by 140 minutes (Sandler et al. 2015). Yet, the kicked cell-cycle toy model (see [figure 3d](#)) aims to explain a cell-cycle difference of 2 hours between mothers (T1) and daughters (T2) (Sandler et al. 2015) by an oscillating internal factor, but the observed pattern seem to just mirror the changing growth conditions during the experiment. Again, a mother cell-cycle cannot overlap in time with a daughter cell-cycle of the same lineage. In contrast to same-generation cell-cycles of sisters/cousins, which reportedly overlapped in time by 94% (Sandler et al. 2015). Hence, there is no need for a fancy theoretical model with an oscillating internal factor to explain the observations. The proposed oscillating internal factor is actually external and is called 'changing growth conditions'.

REFERENCES

Sandler, O., S. P. Mizrahi, N. Weiss, O. Agam, I. Simon, and N. Q. Balaban. 2015. "Lineage correlations of single cell division time as a probe of cell-cycle dynamics." *Nature* no. 519 (7544):468-71. doi: [10.1038/nature14318](https://doi.org/10.1038/nature14318).

APPENDIX

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