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Final words: cell age and cell cycle are unlinked

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Cooper has a simple belief: that the cell cycle is connected to age and size. Furthermore, as a result of this connection in his mind he believes that there are no possible manipulations that can operate on a batch culture to synchronize cells within the cell cycle, such that those cells can undergo a semblance of a normal cell cycle. His formulation of this argument is as a 'fundamental law', the law of conservation of cell-age order (LCCAO). The first part of this law – 'there is no batch treatment of the culture that can lead to an alteration of the cell-age order' – can probably be proved true, in the mathematical sense, and certainly makes intuitive sense. Unfortunately the corollaries of this law are rather suspect, drawing inferences from cell age to cell size to the cell cycle.

Although we are familiar with substantial evidence that allows (but does not require) connections between cell size and the cell cycle (for recent literature on the subject see [1–3]), we know of no corresponding evidence connecting age to the cell cycle. To be clear, we are in some sense arguing at cross-purposes – Cooper argues that cell age is disrupted and we argue that the cell cycle is normal. We have never stated in any forum that by 'synchronized' we mean that all cells are of the same age. Rather, what we have stated is that the population of cells in a whole-culture synchronization are enriched (very strongly in the synchronizations that we have used) around one initial point within the cell cycle and that we are able to use these cell-cycle-synchronized cells to gain substantial insight into how a normal cell cycle proceeds. Cooper argues that any observations we make based on batch-synchronized cultures are inherently due to the method of synchronization. If this were the case, then clearly, we would not expect different methods of synchronization to show similar results when examining the cell cycle. We do not argue that each method shows exactly the same transcriptional profile, we argue that they are similar enough to suggest that we are observing real cell cycle phenomena. Rather than try to derive some value as to how similar the results of one experiment are compared to another, we invite readers to make up their own minds, and look at the graphical representation of the data of Spellman *et al.* [4] (http://genome-www.stanford.edu/cellcycle/figures/figure1A.html) and Whitfield *et al.* [5]. (http://genome-www.stanford.edu/Human-CellCycle/Hela/images/figure3a.html).

Cooper argues in his second Opinion article (this issue, pp. 274–276, DOI:10.1016/j.tibtech.2004.04.011) that whole-culture synchronization methods do not produce a population of cells that reflect the size, DNA content or mitotic configuration, or the physiological state of cells of a particular cell age during the cell cycle of cells growing in steady-state, unperturbed conditions; and he then states that we agree with him. This is misleading – we believe strongly that batch synchronization of cell culture does produce a population of cells that are synchronized with respect to their DNA content, mitotic configuration and physiological state, and further that when the restriction placed on the cells to hold them in this configuration is relieved, that the ensuing cell cycles are by and large reflective of a normal cell cycle. It is because we recognize that restrictive (rather than selective) synchronization methods probably trigger some non-physiological effects that we performed multiple, different experimental procedures. Of course we invite Dr Cooper to use microarrays to analyze the cell cycle transcription of a synchronous yeast cell cycle, using a population of cells generated from a 'baby machine'. If indeed he is able to generate synchronous cell divisions in which there is no evidence of periodic transcription of the genes we believe to be periodically expressed, we will gladly and publicly accept his findings.

Instead of using unfounded arguments masquerading as laws (for instance the suggestion that normal cell division can only be investigated using cells of identical age) we can ask ourselves what evidence exists to support the belief that whole-culture synchronization is an effective tool for studying the cell cycle. In our first article (this issue, pp. 270–273, DOI:10.1016/j.tibtech.2004.04.010) we laid out five points of evidence, which we repeat here: (i) whole-culture synchronized cells undergo the hall-marks of division in similar manners; (ii) genes identified as periodically expressed in whole-culture synchronized cells from different organisms (i.e. humans and yeast) are frequently orthologous; (iii) genes identified as cell-cycle-regulated are frequently found to have a role in the cell cycle, at the point that or shortly after they reach peak expression; (iv) there is an overlap between genes identified as cell-cycle-regulated in whole- and selection-synchronized cultures;

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(v) genes identified as cell-cycle-regulated show cell-cycle-regulation in individual cells.

If one is concrete in one's beliefs, as Cooper is, then no amount of correlation will influence one's opinion. We admit that arguments (ii) and (iii) above are correlative in nature; we do however find them compelling because there is an exceptionally strong link between expression pattern and gene function, which has been found in innumerable biological cases. Cooper's response to our other arguments is, in our opinion, underwhelming. He cannot explain why the functional processes of cell division are not fundamentally disrupted in whole-culture synchronized cells, even in α-factor synchronized yeast in which volumes easily become four times that of unsynchronized cells. Nor can he explain the concordance between whole-culture and batch-culture or whole-culture and single cell observations other than to call them coincidental. He can only point out that there is noise in some systems, or that some experiments are poorly performed, or that whole-culture synchronization induces stresses in the population, all of which we admit. He claims that elutriation of yeast cells should work best, but having performed the experiments we know that the synchrony of these cells was by far the worst. Further we know that these cells are growing in substantially different environmental conditions, with doubling times about six times longer than the other yeast methods, which makes their cell cycles very plausibly different, for we are capable of believing that environmental conditions can influence the cell cycle. Yet we still believe that our elutriation experiment resulted in similar patterns of expression to our other experiments (http://genome-www.stanford.edu/cellcycle/figures/figure1A.html). Try as he might Dr Cooper cannot show in any convincing manner that eukaryotes do not have a substantial number of genes that vary with cell cycle needs.

References

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