Cell death, DNA breaks and possible rearrangements: an alternative view

I thank Gilmore et al.1 for their interest in the work of my colleagues and I on programmed cell death (PCD) and DNA double stranded breaks (DSBs), and in our ideas on neural DNA rearrangements.1 This made for entertaining reading combined with the exciting implications raised by mutations of genes encoding nonhomologous end-joining (NHEJ) proteins. New insights on mice with mutations in other genes for NHEJs (Ref. 2) strengthen the already compelling case for the involvement of NHEJ proteins in neurogenesis. However, perhaps because these topics are far removed from the published expertise of Gilmore et al., several fundamental misconceptions are propagated in their article. In particular, their belief that neuroblast PCD is insignificant and that DSBs are unrelated to PCD mechanisms, and their proposal that a V(D)J-like mechanism could account for our detected DSBs is at odds with the published, experimental literature. Their conclusions are based on several lines of evidence.

Histology

A relative lack of pyknotic nuclei is cited as proof that PCD is not significantly occurring. However, in some of the best examples of PCD, pyknotes are not an accurate measure of the extent of PCD taking place: sparse pyknosis is evident in the thymus, where over 95% of thymocytes die, and also in small intestinal villi, where all cells die every few days.3

Neurogenetic models

Gilmore et al. make numerical arguments against PCD that rely on the validity of the models used.4 If these models are inaccurate then conclusions based on them are uncertain at best. It would seem that the model of Caviness et al.5 is certainly inaccurate as it does not account for neuronal contributions from noncortical regions like the ganglionic eminence (subcortical telencephalon), let alone even consider the operation of PCD. In addition, the problems of drawing temporally valid, mechanistic conclusions about the cell cycle and cell proliferation by comparing different populations of cells in different animals, increases the level of uncertainty. Furthermore, because bromo-deoxyuridine (BrdU) incorporation is not specific for cell proliferation (for example, during DNA repair), the primary data cited by Gilmore et al.6 (from models of Caviness et al.)7 are yet again uncertain. One is left with uncertainties about uncertainties about uncertainties. The numerical arguments raised by Gilmore et al. can easily be addressed. For example, 50% PCD is no problem if death affects subpopulations of cells and thus maintains a sufficient number of proliferating cells to allow for brain growth. Finally, the studies on which the inaccurate models of Caviness et al. are based do not directly address the operation of PCD, and thus, any conclusions drawn are based on indirect inferences.

ISEL+

We have developed and used two independent approaches to study PCD: in situ end-labeling plus, ISEL+ and ‘ligation mediated PCR’ or LMPCR, which identifies nucleosomal ladders associated with apoptosis. Both techniques have been used in our studies and accurately identify dying cells in every tissue known to

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undergo PCD that we examined (these techniques tell us nothing about the clear-
ance time of dead cells). Examples include normal embryo, normal thymus, death-
induced thymus, postnatal retina, Schwann cells in culture and induced death in tissue culture cells, among oth-
ers13–16. Quantitative estimates of the number and occurrence of DSBs required for detection by ISEL+ is of the order of thousands per cell, based on published calculations17. Even if this is an overestimation by a fac-
tor of 10, this still represents hundreds of DSBs per cell. Could a V(D)J-type DNA
rearrangement, as proposed by Gilmore et al. to account for ISEL+ labeling, create
this many DSBs? There is no evidence to support this, as V(D)J recombination is
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deleted or labeled neuroblasts is observed10.

If Gilmore et al. truly believe that there is no significant neuroblast PCD as
they have stated, they should show this by direct experimentation. I continue to favor some form of neural DNA
rearrangements: however, based on available experimental data, it would have to be distinct from V(D)J forms. My views on the role of NHEJ, PCD and possible DNA rearrangements are discussed elsewhere11,12.

**References**


**Reply**

The goal of our article1 was to draw attention to the XRCC4 and ligase IV mutant mice as providing evidence for the possible existence of DNA double strand breaks (DSBs), and to the possibility of DNA recombination in proliferating cells in the developing brain2–4. We also suggested, as a secondary point, that the existence of larger numbers of DSBs than previously suspected might explain the incongruity between different methods of estimating the levels of cell death in the proliferative neuroepithelium.

Chun maintains that his studies using the ISEL+ (in situ end labeling plus) method establish levels of cell death in the