APPENDIX

Estimation of the Ratio (γ) of Poly(ADP-Ribose) Synthetase Activity in Non-Lower-Crypt Cells to that in Lower-Crypt Cells *in vivo* from an Analysis of Enzyme Activities and Thymidine Incorporation in Populations of Sequentially Isolated Cells

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Suppose $\theta_1, \theta_2, \theta_3, \theta_4$ are the proportions of lower-crypt cells present in the isolated cell populations 1, 2, 3 and 4. I will describe the argument for the comparison of isolated cell populations 1 and 4, as these give most information, but the same argument could be applied to any pair of cell populations.

If thymidine incorporation takes place only in lower-crypt cells *in vivo*, the ratio:

$$\alpha = \frac{\theta_1}{\theta_4} = \frac{[^3\text{H}]\text{DNA in isolated cell population 1}}{[^3\text{H}]\text{DNA in isolated cell population 4}}$$

This ratio, and the corresponding ratio of poly(ADP-ribose) synthetase activity (β), are the only two hard items of statistically useful information to be gained from the data, as the absolute enzyme activities and incorporation values vary from experiment to experiment (Table 1 of the main paper). If we assume that the measured poly(ADP-ribose) synthetase activity is simply the sum of the activities of the two cell types, then in a mixed population of cells:

$$\beta = \frac{\theta_1 + \gamma(1 - \theta_1)}{\theta_4 + \gamma(1 - \theta_4)}$$

where γ is the ratio of poly(ADP-ribose) synthetase activity of non-lower-crypt to that of lower-crypt

cells *in vivo*. Using $\frac{\theta_1}{\theta_4} = \alpha$, we can write:

$$\beta = \frac{\alpha + \gamma \left(\frac{1}{\theta_4} - \alpha \right)}{1 + \gamma \left(\frac{1}{\theta_4} - 1 \right)}$$

whence

$$\gamma = \frac{\theta_4}{\theta_4 + \left(\frac{1 - \beta}{\beta - \alpha} \right)}$$

Putting in the best estimates of α and β from the data in experiments (D)–(G) (Table 1 of the main paper):

$$\gamma = \frac{\theta_4}{\theta_4 + 6.042}$$

Although θ_4 is not known, we can get useful limits on γ by considering the extreme possibilities for θ_4 . For example, θ_4 cannot exceed 1, and we can be sure that it will not be less than 0.5 because the isolated cell population (4) is probably the purest. So we get $0.076 < \gamma < 0.142$. This shows that even crude information about θ_4 enables us to get useful knowledge about γ .

In some experiments we have, in addition, information about the ratio:

$$\frac{\text{Phosphatase activity in isolated cell population 1}}{\text{Phosphatase activity in isolated cell population 4}} = \delta$$

Since independent evidence shows that alkaline phosphatase activity in lower-crypt cells is zero, or very close to zero, *in vivo*:

$$\delta = \frac{1 - \theta_1}{1 - \theta_4}$$

Had the alkaline phosphatase activity, thymidine incorporation and poly(ADP-ribose) synthetase activity all been determined on each population of isolated cells, the value for γ would have been determinate. Under these circumstances we have:

$$\alpha = \frac{\theta_1}{\theta_4}$$

and

$$\delta = \frac{1 - \theta_1}{1 - \theta_4}$$

giving the solution

$$\theta_4 = \frac{\delta - 1}{\delta - \alpha}$$

Hence

$$\gamma = \frac{\frac{\delta - 1}{\delta - \alpha}}{\left(\frac{\delta - 1}{\delta - \alpha} \right) + \left(\frac{1 - \beta}{\beta - \alpha} \right)}$$

Thus had all three assays of Table 1 of the main paper been performed on the same isolated cell

Table 1. Values for α , β and δ calculated from results shown in Table 1 of the main paper

Mean activity	Alkaline phosphatase activity in isolated cells <i>in vitro</i> (units/mg of protein)	[³ H]Thymidine incorporation into DNA <i>in vivo</i> (c.p.m./mg of protein)	Poly(ADP-ribose) synthetase activity in isolated nuclei <i>in vitro</i> (units/mg of protein)
Isolated cell population 1	2.6838	2682	12.639
Isolated cell population 4	0.4031	20250	49.449
(Logarithmic means ratio)	$\delta = 6.6573$	$\alpha = 0.1324$	$\beta = 0.2556$

populations (1) and (4) we could calculate the values of α , β and δ shown in Table 1 of this Appendix. So the ratio:

been. It should be noted that the value of γ has been shown [without the aid of the analyses from experiments (A)–(C) in Table 1 of the main paper] to lie

$$\begin{aligned} \gamma &= \frac{\text{poly(ADP-ribose) synthetase activity in population 1 *in vivo*}}{\text{poly(ADP-ribose) synthetase activity in population 4 *in vivo*}} \\ &= \frac{0.86703}{0.86703 + 6.0422} = 0.125 \end{aligned}$$

Even though the value of δ has been calculated from analyses performed in experiments (A)–(C) of Table 1 of the main paper, and the values of α and β have been derived from analyses performed in independent experiments (D)–(G) of Table 1 of the main paper, the value of $\gamma = 0.125$ just calculated is not invalid, simply less accurate than it could have

between 0.076 and 0.142; it is obvious that the precise value of δ makes surprisingly little difference to the estimate of γ . Hence the value $\gamma = 0.125$ is probably quite a good estimate, but it would be straining the analysis too far to assign confidence limits for the estimated value of γ .