full-length transcript as control cells. Cells from SMA type I and II individuals produced 27% and 21% more alternative transcript, respectively, than control samples, whereas SMA III cells produced 54% less alternative transcript than controls. Thus, the ratio of alternative to full-length transcripts is significantly higher in types I and II than that of type III SMA. In summary, we observed a correlation between SMN transcription patterns and SMA phenotypes. SMA type I and II individuals appeared to produce significantly more alternative transcript than SMA type III individuals. As alternative SMN transcripts are generated only from *SMN2*, our findings suggest a pathogenic role for *SMN2*. We have attempted to associate the SMN alternative transcripts with their *SMN2* copy numbers in each SMA subtype, but found no correlation between these two measurements (data not shown). The SMN transcription pattern therefore appears to be an independent factor that influences SMA phenotypic severity. It is possible that the alternative transcript may interfere with the normal function of the intact SMN transcript through competitive binding to its substrates. Alternatively, a truncated SMN protein, which has been observed in the mammalian central nervous system[14], may be derived from this alternative transcript and interfere with the intracellular function of the intact SMN protein. This hypothesis is consistent with the recent report that an SMN protein isoform (lacking exon 7) has a reduced capacity to self-associate[15].

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DRPLA aggregation and transglutaminase, revisited

Cells that express proteins with long amino-terminal stretches of glutamines, such as those employed by Igarashi *et al.*[1], should be useful for investigating the question of whether crosslinking by transglutaminase[2], might cause precipitation of pathological proteins of this type, and whether the enzyme is responsible for the induction of apoptosis. However, in the absence of proper controls[3], the study by Igarashi *et al.* still leaves the issue unresolved.

The authors' claims are based on the effects of cystamine[4] and monodansylcadaverine[5] (MDC), two compounds that can be used to probe the reactions of transglutaminases. Cystamine is known to inactivate the enzyme[3], probably by forming a mixed disulfide. This raises the question of whether similar interference with other thiol enzymes in COS cells (such as the caspases) might also occur. As primary amines, both cystamine and MDC are good substrates for transglutaminase[6], and are useful for blocking the glutamine residues in proteins which would otherwise participate in forming N$^{\varepsilon}$(γ-glutamyl)lysine bridges. However, important hurdles must be overcome before making a reasonable case for suggesting that these substrates might specifically inhibit crosslinking by transglutaminase in a cellular setting. Precautions should be taken to inhibit oxidases[3,7] so as to prevent possible conversions of the substrates to aldehydes, which would certainly modify a variety of cell constituents. To prove that the transglutaminase-reactive, primary amino group in MDC was needed for inhibition, a minimal requirement would be to compare the effects of MDC with those of its dimethylated tertiary amine analogue[3,7]. Of course, direct labelling of the DRPLA protein by MDC should also be provided[7–9]. In addition, it is important to bear in mind that the chemical structure of MDC is quite similar to compounds (W5, W7) that are widely marketed as calmodulin inhibitors, raising the possibility that MDC might interfere with the functions of calmodulin in COS cells. It was rather puzzling to find in the article of Igarashi *et al.* that MDC prevented nuclear fragmentation, that is, apoptotic cell death (Fig. 8*e*), but was ineffective in blocking the formation of aggregate bodies supposedly produced by the transglutaminase-catalysed crosslinking of the DRPLA protein (Fig. 8*b*).

Howard Green's original suggestion[2]—not just as an alternative, but perhaps in concert with the polar zipper[10,11] mechanism of precipitation—still remains an attractive and viable possibility. It may very well turn out that the reaction of transglutaminases with the N-terminal polyglutamine extensions in the DRPLA protein, and also in the other abnormal gene products of related disorders[12], has a role in the development of the neurodegenerative process. Further experiments with COS cells expressing the truncated DRPLA protein could help to examine the validity of this notion.

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