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The assay that defines desmosome hyper-adhesion

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To the Editor

In a recent Letter to the Editor it was suggested that desmoplakin (DP) regulates desmosome hyper-adhesion (Hobbs and Green, 2011). The term hyper-adhesion refers to the strong, calcium-independent adhesive state adopted by desmosomes in tissues, including the epidermis (Garrod *et al.*, 2005; Wallis *et al.*, 2000). This contrasts with a weaker adhesive state adopted by desmosomes on initial formation in tissue culture, in wound edge epithelium and in early embryonic development (Garrod, 2010; Garrod *et al.*, 2005; Kimura *et al.*, 2007; Thomason *et al.*, 2012; Wallis *et al.*, 2000). Desmosomal hyper-adhesion appears to be important for the integrity of the epidermis as indicated by its role in wound healing and pemphigus (Cirillo *et al.*, 2010; Garrod *et al.*, 2005; Thomason *et al.*, 2012).In order to show that desmosomes are hyper-adhesive a specific assay for calcium independence is required.

The adhesion molecules of desmosomes, desmocollin (Dsc) and desmoglein (Dsg), are cadherins, a protein family widely involved in calcium dependent cell-cell adhesion. In desmosomal hyper-adhesion Dsc and Dsg appear to become locked into a quasi-crystalline configuration, which gives rise to the characteristic intercellular midline structure seen in electron micrographs of tissue desmosomes (Al-Amoudi *et al.*, 2007; Garrod *et al.*, 2005; He *et al.*, 2003). Long experience has shown that hyper-adhesive desmosomes are truly calcium independent in experimental assays. This means that they resist calcium chelation by agents that reduce the extracellular calcium concentration to the nanomolar range (Garrod *et al.*, 2005; Mattey and Garrod, 1986; Wallis *et al.*, 2000; Watt *et al.*, 1984). We have suggested that this may be because calcium becomes locked into the quasi-crystalline arrangement adopted by the desmosomal cadherins (Garrod *et al.*, 2005).

DP is a major desmosome plaque component essential for binding between the plaque and intermediate filaments. This linkage is crucial for tissue integrity and the cohesive strength of cell sheets (Garrod and Chidgey, 2008; Green and Simpson, 2007; Huen *et al.*, 2002; Vasioukhin *et al.*, 2001).The suggested role of DP in regulating hyper-adhesion was based on the observation that adhesion was enhanced by expressing a DP point mutation (Ser2849Gly) in A431 cells. This mutation enhances the intermediate filament binding of DP by 9-fold compared with wild-type DP (Meng *et al.*, 1997). Hobbs and Green found that detached sheets of cells expressing DP Ser2849Gly were more resistant to mechanical disruption than wild-type cells after exposure to low calcium medium for 45 minutes (Hobbs and Green, 2011). Furthermore, hyper-adhesion can be converted to calcium dependent adhesion by activation of protein kinase C by phorbol ester (Wallis *et al.*, 2000). Cells expressing DP Ser2849Gly were not susceptible to such conversion to calcium dependence,

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further supporting the posited role for DP in regulating hyper-adhesion (Hobbs and Green, 2011).

While the results of Hobbs and Green elegantly show that expression of Ser2849Gly enhances the cohesiveness of cell sheets, they do not enable any conclusion to be drawn regarding hyper-adhesion. This is because the assay used in the adhesion experiments does not enable one to determine whether or not the cells were hyper-adhesive. Hyper-adhesion is defined by resistance of desmosomes to disruption by calcium chelation. The low calcium medium used for assaying hyper-adhesion consists of calcium-free DMEM plus 10% chelated foetal bovine serum plus 3 mM EGTA (see (Garrod *et al.*, 2005; Kimura *et al.*, 2007; Wallis *et al.*, 2000)). Furthermore, exposure to such a medium for a minimum of 90 minutes is required to confirm hyper-adhesion. The low calcium medium used by Hobbs et al. was reportedly DMEM, 10% foetal bovine serum, 1% penicillin/streptomycin, 0.05 mM Ca^{2+}. Thus the concentration of calcium used in the assay was between five and six orders of magnitude greater than that required to define hyper-adhesion. Furthermore, while it is likely that prolonged culture of cells in the medium used by Hobbs and Green would cause cells to down-regulate their desmosomes, the 45 minute exposure to such a medium used in their experiments is insufficient to define hyper-adhesion.

I think confusion may arise because so-called “calcium switching” is used to study both the assembly of desmosomes and desmosome disruption. These are two quite distinct processes, requiring different assays. Physiological extracellular calcium concentration is of the order of 1mM. However, many epithelial cell types can be grown in media containing one tenth of this amount of calcium, or even less. Under these conditions they do not assemble intercellular junctions. If the calcium concentration is then raised to a physiological level, the cells rapidly assemble junctions (Hennings and Holbrook, 1983). I assume that if such cells are returned to the original low calcium medium, desmosomes will gradually disappear, though I am not aware that this has been systematically studied. However, if the intention is to study desmosome disruption it is usual to induce rapid loss if desmosomal adhesion, not simply be adding low calcium medium, but by chelating calcium through the addition of EGTA. Under these conditions, newly assembled, calcium dependent desmosomes have been shown to lose adhesion within 15 minutes (Mattey and Garrod, 1986). By contrast, hyper-adhesive desmosomes resist such treatment for hours (Garrod *et al.*, 2005). The use of EGTA is therefore essential to determine whether or not desmosomes are hyper-adhesive.

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Abbreviation

DP	desmoplakin
Dsc	desmocollin
Dsg	desmoglein
DMEM	Dulbecco’s minimum essential medium
EGTA	ethylene glycol-bis (β-aminoethyl ether)-N,N,N’,N’-tetraacetic acid

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