Structure and Assembly of Desmosome Junctions: Biosynthesis and Turnover of the Major Desmosome Components of Madin–Darby Canine Kidney Cells in Low Calcium Medium

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Abstract. Neither stratifying (primary keratinocytes) nor simple (Madin–Darby canine kidney [MDCK] and Madin–Darby bovine kidney [MDBK]) epithelial cell types form desmosomes in low calcium medium (<0.1 mM), but they can be induced to do so by raising the calcium level to physiological concentrations (standard calcium medium [SCM], 2 mM). We have used polyclonal antisera to the major bovine epidermal desmosome components (>100 kD) in a sensitive assay involving immunoprecipitation of the components from metabolically labeled MDCK cell monolayers to investigate the mechanism of calcium-induced desmosome formation.

MDCK cells, whether cultured in LCM or SCM, were found to synthesize the desmosome protein, DPI and desmosome glycoproteins DGI and DGII/III with identical electrophoretic mobility, and also, where relevant, with similar carbohydrate addition/processing and proteolytic processing. The timings of these events and of transport of DGI to the cell surface were similar in low and high calcium. Although the rates of synthesis of the various desmosome components were also similar under both conditions, the glycoprotein turnover rates increased dramatically in cells cultured in LCM. The half-lives decreased by a factor of about 7 for DGI and 12 for DGII/III and, consistent with this, MDCK cells labeled for 48 h in SCM had three and six times the amount of DGI and DGII/III, respectively, as cells labeled for 48 h in LCM. The rate of turnover and the levels of DPI were changed in the same direction, but to much lesser extents. Possible mechanisms for the Ca\(^{2+}\)-dependent control of desmosome formation are discussed in the light of this new evidence.

One of the major cell–cell adhesion structures in epithelial tissues is the desmosome junction. The biochemical composition of desmosomes is well documented for preparations isolated from bovine muzzle stratum spinosum and the nomenclature used for components from other sources is based on this system, as discussed in Penn et al. (1987). The nonglycosylated desmosome proteins (DP) are: DPI, 250 kD; desmocalmin, a minor calmodulin-binding protein, 240 kD; DPII, 215 kD; DPIII, 83 kD; DPIV, 75 kD. The desmosome glycoproteins (DG) are: DGI, 150 kD; DGII, 120 kD; DGIII, 100 kD; DGIV, 22 kD. A close relationship between DGII and DGIII has been shown by peptide mapping (Cohen et al., 1983). All these components have either been localized to the desmosome by immunoelectron microscopy (Cohen et al., 1983). All these components have either been localized to the desmosome by immunoelectron microscopy (Cohen et al., 1983; Franke et al., 1983b; Gorbsky et al., 1985; Franke et al., 1987; Steinberg et al., 1987; Miller et al., 1987) or shown to copurify with desmosomes (Gorbsky and Steinberg, 1981; Gorbsky et al., 1985; Tsukita and Tsukita, 1985).

1. Abbreviations used in this paper: DG, desmosome glycoprotein; DP, desmosome protein; LCM, low calcium medium; SCM, standard calcium medium; MDBK, Madin–Darby bovine kidney.

Using antibodies to bovine muzzle desmosome components, cross-reactive epitopes have been identified in a wide variety of cell types and species by immunofluorescence microscopy (Franke et al., 1981, 1982, 1983; Cowin and Garrod, 1983; Cowin et al., 1984). More recent studies on desmosome-enriched preparations from other tissues and cultured epithelial cells using immunoblotting or immunoprecipitation techniques have confirmed similarities between the components from different sources, especially with DPI and DGI and to a lesser extent also with DGII/III. DPIII appears to be restricted to stratifying cell types (Franke et al., 1982; Cowin et al., 1985; Penn et al., 1987) though results are conflicting (Giudice et al., 1984; Suhrbier and Garrod, 1986). Several groups have identified a DGI-like polypeptide of molecular mass close to 150 kD in cells from a wide variety of different species and tissues (Giudice et al., 1984; Suhrbier and Garrod, 1986; Schmelz et al., 1986; Penn et al., 1987). Using monoclonal antibodies, both highly conserved (Schmelz et al., 1986) and tissue-specific epitopes have been identified for DGI (Giudice et al., 1984). DGII and III have likewise been identified in a variety of different cell types, including simple epithelia, by polyclonal antisera.
and that events leading to desmosome formation can be separated from those required for stratification. The reverse process of desmosome disruption can be brought about by Ca\(^{2+}\) depletion (Kartenbeck et al., 1982; Mattey and Garrod, 1986b), which may also affect other junction types, namely tight junctions as in mouse mammary epithelial cells and guinea pig acinar cells (Pitelka et al., 1983; Meldolesi et al., 1978) and adhaerens junctions as in MDBK cells (Volberg et al., 1986). Using a sensitive assay combining the metabolic labeling of cells in culture with the immunoprecipitation of labeled desmosome polypeptides by polyclonal antisera raised in guinea pigs against the desmosome components of bovine muzzle epidermis exactly as described by Penn et al. (1987). Antiserum were used as previously characterized and were named according to the bands recognized in bovine nasal epidermal desmosomes: anti-DPI/II; anti-DGI; anti-DGII/III. Immunoprecipitated proteins were released by boiling (5 min) in sample buffer containing 100 mM dithiothreitol and analyzed by SDS-PAGE using an acrylamide concentration of 7.5% (Laemmli, 1970). Gels were treated for fluorography, dried, and exposed to prefwalked Kodak XAR5 film for 3–21 d (Bonner and Laskey, 1974). Where necessary the amount of a particular desmosome component present was determined by scanning the appropriate region of the fluorograph using a densitometer (Chromoscan 3; Joyce, Loebl and Co. Ltd., Gateshead, UK) and expressing the data in arbitrary units.

Total protein was determined by the method of Lowry et al. (1951) including 0.1% SDS in all samples and standards. The incorporation of \(^{35}\)S-methionine into total protein was determined as TCA-precipitable radioactivity.

### Results

#### Biosynthesis of Desmosome Components in MDCK Cells Cultured in SCM and LCM

The time course for calcium-induced desmosome formation was monitored by fixing MDCK cells at various times after replacing SCM with LCM and staining with anti-DPI/II antiserum. As previously reported (Mattey and Garrod, 1986a) when grown in SCM, discrete spots were observed solely at cell boundaries, but in LCM all cells lacked peripheral staining and only cytoplasmic and perinuclear fluorescence was seen. In the MDCK cells used here clear evidence of discrete staining at cell margins was seen at 120 min after switching to SCM and gradually became brighter over the next few hours (data not shown). During this period, cytoplasmic and perinuclear staining diminished. This time course was confirmed by thin section electron microscopy (data not shown).

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### Materials and Methods

#### Cell Culture

MDCK cells of the high passage number, low resistance type (Richardson et al., 1981) were obtained from the European Collection of Animal Cell Cultures, Public Health Laboratory Service Centre for Applied Microbiology & Research, Porton Down, Salisbury, Wilt., United Kingdom, and were maintained as previously described (Penn et al., 1987) in MEM, 10% (vol/vol) FCS, 0.006% (wt/vol) penicillin, 0.001% (wt/vol) streptomycin (SCM). For experiments, flasks of confluent cells were divided into 35- or 50-cm\(^2\) plastic dishes such that confluent monolayers were reestablished after 16–24 h. For low calcium cultures, cells were switched to LCM after ~6 h in SCM and allowed to grow in LCM for 16–24 h before experimentation. LCM had the same formulation as MEM except that calcium salts, with the exception of calcium pantothenate, a very minor component, were omitted and the FCS had been depleted of divalent cations by dialysis and chelating-resin treatment (Brennan et al., 1975) using Chelex 100 resin (Bio-Rad Laboratories Ltd., Watford, Hertshire, UK). The calcium concentration of SCM was ~2 mM and that of LCM was <0.1 mM as determined by atomic absorption spectroscopy.

#### Metabolic Labeling of Cells and Immunoprecipitations

Metabolic labeling of cell monolayers was essentially as described by Penn et al. (1987). For short labeling periods (10 min to 3 h), cells were preincubated for 30 min in the appropriate methionine-free medium, then labeled in the same medium containing 100 \(\mu\)Ci/ml \(^{35}\)S-methionine (sp act >800 Ci/mmol; Amersham International, Aylesbury, Bucks, UK) for 1–3 h or 1 mCi/ml for 10 min. To label for 48 h, cells were incubated with the appropriate standard concentrations of calcium (SCM; 1–2 mM). This was first used with mouse keratinocytes when it was found that the cells proliferate and migrate rapidly in LCM, but do not stratify and are separated by wide intercellular spaces. On raising the calcium concentration of the culture medium cell contact occurs, desmosomes form, cells stratify, and finally cornification takes place (Hennings et al., 1980; Hennings and Holbrook, 1983; Watt et al., 1984; Magee et al., 1987). These changes mimic the orderly differentiation of keratinocytes in vivo. Similar calcium switch experiments have also been performed to induce desmosomes in simple epithelial cell lines such as Madin–Darby canine kidney (MDCK), Madin–Darby bovine kidney (MDBK), and a rat mammary epithelial line (Bologna et al., 1986; Mattey and Garrod, 1986a). These systems have the advantages of being established cell lines and that events leading to desmosome formation can be separated from those required for stratification. The reverse process of desmosome disruption can be brought about by Ca\(^{2+}\) depletion (Kartenbeck et al., 1982; Mattey and Garrod, 1986b), which may also affect other junction types, namely tight junctions as in mouse mammary epithelial cells and guinea pig acinar cells (Pitelka et al., 1983; Meldolesi et al., 1978) and adhaerens junctions as in MDBK cells (Volberg et al., 1986). Using a sensitive assay combining the metabolic labeling of cells in culture with the immunoprecipitation of labeled desmosome polypeptides by polyclonal antisera raised in guinea pigs against the desmosome components of bovine muzzle epidermis exactly as described by Penn et al. (1987). Antiserum were used as previously characterized and were named according to the bands recognized in bovine nasal epidermal desmosomes: anti-DPI/II; anti-DGI; anti-DGII/III. Immunoprecipitated proteins were released by boiling (5 min) in sample buffer containing 100 mM dithiothreitol and analyzed by SDS-PAGE using an acrylamide concentration of 7.5% (Laemmli, 1970). Gels were treated for fluorography, dried, and exposed to prefwalked Kodak XAR5 film for 3–21 d (Bonner and Laskey, 1974). Where necessary the amount of a particular desmosome component present was determined by scanning the appropriate region of the fluorograph using a densitometer (Chromoscan 3; Joyce, Loebl and Co. Ltd., Gateshead, UK) and expressing the data in arbitrary units.

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Comparison of the desmosome components synthesized by MDCK cells cultured in SCM and LCM. Confluent monolayers of MDCK cells were prepared in SCM (+) or LCM (−) as described in Materials and Methods. Cell lysates were immunoprecipitated using anti-DP (lanes 1 and 2), anti-DGI (lanes 3–6), or anti-DGII/III (lanes 7–10) antisera using cells pulse labeled with [35S]methionine for 10 min, then chased for 0 min (P), or 2 h (C) as described in Materials and Methods. The precursor form of each DG is marked by the subscript O. The position of migration of molecular mass markers (●), given in kilodaltons, was determined by Coomassie Blue staining of the gel after fluorography. Bands in the lower portion of the fluorograph are keratin polypeptides nonspecifically precipitated.

The major high molecular mass desmosome components (>100 kD) synthesized by MDCK cells in LCM are shown in Fig. 1 (indicated by −). The components synthesized by MDCK cells cultured in medium containing standard levels of calcium are also shown (Fig. 1, indicated by +; see also Penn et al., 1987). The bovine muzzle anti-DPI/II antiserum immunoprecipitated a major polypeptide of equivalent mobility to the epidermal DPI whether cells were cultured in SCM (Fig. 1, lane 1) or LCM (lane 2). Under both culture conditions, faster migrating polypeptides were also observed in lesser amounts than DPI. These probably corresponded to proteolytically degraded DPI or small amounts of DPII (see Penn et al., 1987 for discussion). Similarly for both culture conditions, the DPI polypeptides synthesized in a 10-min pulse (Fig. 1, lanes 3 and 4) and 2-h chase (lanes 5 and 6) had identical electrophoretic mobilities. The slight decrease in mobility of DPII observed after a 2-h chase (Fig. 1, lanes 5 and 6) is thought to be due to the processing of the high mannose cores, added co-translationally to the polypeptide, to complex oligosaccharide chains (Penn et al., 1987). That this was observed for DPII synthesized by cells cultured in LCM would suggest that carbohydrate addition and processing was not substantially impaired by such culture conditions. This was confirmed by treating cells with tunicamycin, which reduced the polypeptide of molecular mass identical in both LCM and SCM (by 5–10 kD, as shown in Penn et al., 1987 for high calcium). The electrophoretic mobility of the DGII/III polypeptides were identical whether immunoprecipitated from cells cultured in SCM or LCM (Fig. 1, lanes 7–10). In both cases, the two polypeptides were synthesized concurrently as discrete polypeptides (Fig. 1, lanes 7 and 8) which then underwent similar proteolytic processing (lanes 9 and 10; see Penn et al., 1987). As with DPI, tunicamycin effect identical shifts from newly synthesized and mature DGII/III under both culture conditions (data not shown).

The timing of transport of DGI and posttranslational processing of DGII/III were compared in cells cultured in LCM relative to SCM. Fig. 2a shows that the timing of proteolytic processing of DGII/III was similar under both culture conditions (being 90% complete within 1 h) with possibly a slight lag (15 min) for cells in LCM. The proteolytic processing step is thought to occur during the transport of the DGII/III.
polypeptides to the cell surface though its significance is not yet understood (Penn et al., 1987). The transport of newly synthesized DGI to the cell surface was also investigated (Fig. 2 b) by probing the accessibility of DGI to trypsin in intact cells, when the major cleavage product could be assayed by immunoprecipitation and SDS-PAGE (see Penn et al., 1987). The rate of migration of DGI to the cell surface in cells cultured in LCM was similar for cells cultured in SCM; although only 80% of the DGI became accessible to trypsin even after long chase periods. The size of the protected fragment was also found to be the same as previously reported (Penn et al., 1987) under both culture conditions (data not shown), consistent with general similarities in membrane orientation and presentation. We conclude that cells in LCM are not substantially impaired in their pathways for biosynthesis and processing of the desmosomal components studied nor in the timing of these events.

**Synthesis of Desmosome Components Is Similar, but Turnover Is Greatly Increased in LCM**

Over the time course of this experiment the rate of total protein synthesis measured as TCA-precipitable radioactivity was linear and similar for cells cultured in SCM or LCM (Fig. 3 a). The apparent rates of synthesis of the individual desmosome components assayed were also similar under both culture conditions (Fig. 3 b). Table I shows the mean of three separate determinations. Although each component appeared to be synthesized at a different rate from the others, we cannot assess the contributions to this from differences in efficiency between the polyclonal antisera at immunoprecipitating their respective antigens after denaturation by boiling in SDS and from variation in the methionine content of the proteins. As direct comparisons could not be made between different antisera, it was not possible to calculate the relative molecular amount of each desmosome component.

Differences were seen between SCM and LCM in relative turnover rates of certain desmosome components (Figs. 4 and 5; summarized in Table I). Total protein turnover was very slightly faster for cells cultured in LCM compared with SCM (Fig. 5 A) and was curvilinear indicating heterogenous turnover rates of individual proteins. In LCM, the turnover of DPI was slightly faster than that in SCM (Fig. 4, lanes 1-4; Fig. 5 B), but for all three DGs a much more dramatic increase in turnover was observed in LCM relative to SCM (Fig. 4, lanes 5-12; Fig. 5 C). Pulse-chase experiments showed little or no trace of the DGs after an 8-h chase in LCM (Fig. 4, lanes 8 and 12), whereas for cells grown in SCM little turnover was in evidence at this time (lanes 7 and 11) although proteolytic processing of DGII/III had occurred. Quantitative measurements of half-lives were made (Fig. 5, B and C show a single experiment and the mean of three separate experiments is given in Table I). In SCM, newly synthesized DGs were found to turnover at a similar rate ($t_{1/2} \sim 20$ h for both; Fig. 5 C), but faster than DPI ($t_{1/2} \sim 60$ h in Fig. 5 B). The turnover rates in LCM (Fig. 5 C and Table I) for DGII/III polypeptides ($t_{1/2} = 1.7$ h) were even faster than for DGI ($t_{1/2} = 2.7$ h). For DPI, the mean relative $t_{1/2}$ was 52 h in SCM compared with 22 h in LCM (Table I). Evidently the glycoproteins are selectively degraded in cells in LCM.

**Long Term Levels of Desmosome Components in Cells Cultured in SCM and LCM**

The similar rates of synthesis of the DGs in SCM and LCM compared with much faster degradation in the latter medium would suggest that the relative levels should differ. This was indeed found to be the case (Fig. 6 and summarized in Table I); the faster turnover of DGII/III relative to DGI in cells cultured in LCM was reflected in that, after 48 h of labeling,
cells in SCM had approximately three times as much DGI and six times as much DGII/III as in LCM (Table I). Relative levels of DPI differed less substantially between SCM and LCM (1.4 times more in the former), in agreement with the more modest threefold decrease in half-life.

**Discussion**

The MDCK cell line used in these studies was found, by immunofluorescence and EM in thin section, to take ~2 h for the assembly of morphologically distinct desmosomes after

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**Figure 4.** Turnover of DPI, DGI, and DGII/III by cells cultured in SCM or LCM. Duplicate dishes of cells in SCM (+) or LCM (−) were pulse labeled for 10 min then chased for 0 (lanes 1, 3, 5, 6, 9, 10), 20 (lanes 2 and 4), or 8 (lanes 7, 8, 11, and 12) h as described in Materials and Methods. Cell lysates (equal protein) were immunoprecipitated with anti-DP (lanes 1–4), anti-DGI (lanes 5–8), or anti-DGII/III (lanes 9–12) antisera. Molecular mass markers are as indicated.

**Figure 5.** Turnover of DPI, DGI, and DGII/III by cells cultured in SCM or LCM. (A) Total protein turnover under both culture conditions was determined by pulse labeling for 1 h and chasing for times shown: SCM (○); LCM (○). (B) Cells were pulse labeled for 1 h and chased for indicated times. (C) Cells were pulse labeled for 1 h in SCM (○, ●), or 10 min in LCM (○, □), and chased as indicated. Pulse times were chosen so as to be short relative to the half-lives. Cell lysates were immunoprecipitated with the appropriate antiserum and analyzed by SDS-PAGE. The amount of each component was quantified as in Materials and Methods. Results were plotted as logₐ (％ of each component remaining) in arbitrary units of area versus chase time in hours.
Figure 6. Relative levels of desmosome components in cells cultured in SCM and LCM. Duplicate dishes of cells were labeled for 24 h with [35S]methionine, then fresh medium and label were added for a further 24 h using either SCM (+) or LCM (−) (see Materials and Methods). Cell lysates were immunoprecipitated with each antiserum and the amount of each component quantified as in Materials and Methods. Results were corrected for total incorporation of TCA-precipitable radioactivity for both culture conditions.

The inability of MDCK cells cultured in LCM to form desmosomes could not be correlated with any change in the synthesis or processing of the protein or glycoprotein constituents that we studied or in the rate of transport of DGI to the plasma membrane. We cannot, however, rule out the possibility that other desmosome components are absent or modified or that there are subtle undetected modifications in the carbohydrate moieties or phosphorylation of the DGs under these conditions. Data have been presented for DGII/III combined due to uncertainty in assigning the identity of their precursors; however, similar data are obtained if DGII and III are treated separately. If changes in the carbohydrate chains of the DGs had occurred on culturing cells in LCM then they were insufficient to alter their mobility on SDS-PAGE. Although we are currently studying the carbohydrate chains on the DGs of bovine muzzle and those synthesized by cells in SCM, it is not possible to compare them directly with those present on DGs synthesized by cells in LCM due to the greatly reduced amounts of glycoproteins under the latter conditions.

Using immunofluorescence microscopy, a technique that is intrinsically nonquantitative and subjective, other groups have detected the major desmosome components in low calcium keratinocytes and MDCK cells (Watt et al., 1984; Jones and Goldman, 1985; Mattey and Garrod, 1986a), but not in MDBK cells (Mattey and Garrod, 1986a). We were able, by immunoprecipitation, to detect DPI, DGI, and DGII/III in MDBK cells cultured in LCM (data not shown); this may reflect the greater sensitivity of our assay or differences in the cell line and culture conditions used. We have also detected desmosome components of equivalent electrophoretic mobility in human keratinocytes and SV-40 transformed keratinocyte cell line (SVK14) cells grown in SCM or LCM (data not shown). An advantage of our experimental approach over immunofluorescence microscopy is that it allows assay of components synthesized in LCM without interference from remnants of desmosomes internalized during cell passing in SCM (Kartenbeck et al., 1982; Mattey and Garrod, 1986a).

A major conclusion from this study was that blockade of desmosome formation by MDCK cells in LCM was associated with accelerated degradation of the DGs. This provides a possible explanation for the finding that cycloheximide treatment of MDCK cells either several hours before switching (Mattey and Garrod, 1986a; our unpublished data) or even, for our cells, concurrently with the switch (our unpublished data) was able to inhibit desmosome formation. Perhaps the pool of desmosome components was insufficient in LCM and de novo protein synthesis was required to permit desmosome formation. The same was not true for primary keratinocytes which form desmosomes on switching to SCM in the absence of protein synthesis (Hennings and Holbrook, 1983; our unpublished data).

The first-order turnover kinetics observed (see Fig. 5, B and C) are consistent with the presence of a homogeneous major pool of each component. This provides no evidence for an assembly mechanism analogous to other multi-subunit complexes; for example the erythrocyte membrane cytoskeleton, enveloped viruses, and the acetylcholine receptor (reviewed by Lazarides and Moon, 1984) where the individual subunits are synthesized in ratios considerably different from those found at steady state in the assembled complex and excess unassembled subunits are rapidly degraded. The different degradation rates exhibited by the two pools might be expected to be reflected as biphasic turnover kinetics.

It is possible to relate the change in pool size to the altered rate of degradation. In the steady state, the rate of synthesis of a desmosome component equals its rate of degradation; whereas synthesis is a zero order process, degradation, if there is a single pool of each component, might be expected to be first order, which indeed was the result observed. Mathematically this can be expressed:

\[ k_s = [D]k_d, \]

where \( k_s \) and \( k_d \) are the rate constants for synthesis and degradation, respectively, and \([D]\) is the steady state concentration of a desmosome component. For cells cultured in SCM (+) and LCM (−) the equations are:

\[ k_s^+ = [D^+]k_d^+; \]
\[ k_s^- = [D^-]k_d^-; \]

However, the measurements of initial rate of synthesis (Fig. 5 B) suggested for the major desmosome components that \( k_s^+ \) was similar to \( k_s^- \). Hence:

\[ [D^+]k_d^+ \approx [D^-]k_d^-, \]

or the relative steady state concentrations of each component...
are equal to the inverse ratios of their degradation constants or the direct ratios of their half-lives. The ratio of half-lives (SCM/LCM) were: DPI, 2.3; DGI, 7.4; DGII/III, 11.5, consistent with the trend in the ratios of levels measured at 48 h which were 1.4, 3.2, and 5.8, respectively.

A more rigorous treatment avoiding the simplifying assumptions made above starts from the rate equation for change of concentration of any of the three desmosomal components under either set of cell culture conditions:

\[ \frac{d[D]}{dt} = k_e - k_d[D], \]

which by integration between zero time and time \( t \) gives:

\[ \log_e(1-k_d[D]/k_e) = -k_d t \]

or

\[ [D] = k_e(1-e^{-k_d t})/k_d. \]

This equation could be fitted to the data in Fig. 3b by taking the independently measured values of \( k_e \) (Fig. 5) to determine each \( k_d \), leaving \( k_e \) as the only free parameter to be determined for each component under each set of conditions as the value giving the least residual mean square. This model predicted the 48-h values for each ratio \([D^+]/[D^-]\) as, 1.23, 4.13, and 6.29, respectively, in excellent agreement with the measured values cited above, providing further support for our model based on single pools of each component with synthesis and degradation by the simple kinetic schemes postulated. Predicted steady state ratios were 2.01, 5.09, and 7.70. Each \( k_d \) was somewhat greater than the corresponding \( k_e \), apparently by an amount greater than experimental error (20% for DPI, 50% for DGI and DGII/III), but further work would be required to establish this with certainty. It is not possible to determine absolute rates of synthesis or degradation with the current information, without knowing the specific activity of the methionine pool, the methionine content of each protein, and the efficiency of immunoprecipitation.

The turnover rates for the desmosome glycoproteins were quite rapid even in SCM. Perhaps this reflects a dynamic character for desmosomes in cell culture (compare Dulbeco et al., 1984 and Ducibella et al., 1975). However, the DGIs were found to turn over faster than the DPs, arguing that the effects are not to be explained in terms of the coordinated degradation of desmosomes as distinct units. In support of this, MDBK cells dissociated with EGTA were found to have the desmosome plaque maintaining its membrane association during internalization (Kartenbeck et al., 1982) whereas DGII/III remained at the cell surface (Cowin et al., 1984b).

The subcellular location of the desmosome components in cells grown in LCM has been suggested to differ from that in normal cells on the basis of immunofluorescence microscopy in keratinocytes and MDCK cells. DGII and III were found by Watt et al. (1984) and Matthey and Garrod (1986a) to be all over the cell surface whereas the other components were diffuse throughout the cytoplasm. We found DGI on the cell surface in LCM, but cannot say if the same is so for DGII/III as the inability to assign tryptic fragments unambiguously prevents us from distinguishing trypsinization of DGII/III from its turnover. Matthey and Garrod (1986a) found by immunofluorescence microscopy that cell surface DGII/III in cells cultured in LCM was more easily extracted by detergent than DGII/III in cells grown in SCM, suggesting that it was not attached to the cytoskeleton in the former situation. The same was not found for DPI/II; Jones and Goldman (1985) detected a preformed DP-cytokeratin complex under both culture conditions and proposed that calcium triggered rapid movement of this complex from the nucleus to the cell periphery, to regions of cell contact.

In conclusion, the only major change we have been able to detect in the biochemistry of desmosome components when MDCK cells are cultured in LCM to arrest junction assembly is in the rate of metabolic turnover. For the glycoprotein components, this represents a very dramatic enhancement of degradation and decrease in relative levels. They are synthesized and transported to the plasma membrane in apparently similar manner as in SCM in which the cells are desmosome competent. We conclude that the level at which the blockade operates on desmosome formation involves the assembly of morphologically recognizable desmosomes after arrival of the three glycoproteins at the plasma membrane. It would seem likely that, in the absence of such assembly, the components are susceptible to accelerated proteolysis. On the evidence presented here, the converse explanation cannot be ruled out for MDCK cells, that the smaller pool size of the glycoproteins caused by increased degradation is below the threshold for assembly. Since other epithelial cells subject to an apparently similar blockade can, however, proceed to desmosome formation when the Ca\(^{2+}\) is elevated in the presence of cycloheximide, this would seem less likely as the basis of a general explanation.

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