The EMBO Journal vol.4 no.13A pp.3393-3398, 1985

# Identification of a putative cell adhesion domain of uvomorulin

**Dietmar Vestweber and Rolf Kemler**

Friedrich-Miescher-Laboratorium der Max-Planck-Gesellschaft, Spemannstr. 37-39, 7400 Tübingen, FRG

Communicated by K.Simons

**A rat monoclonal antibody (DECMA-1) selected against the murine cell adhesion molecule uvomorulin blocks both the aggregation of mouse embryonal carcinoma cells and the compaction of pre-implantation embryos. However, decompacted embryos eventually become recompacted in the presence of DECMA-1 and form blastocysts composed of both trophectoderm and inner cell mass. DECMA-1 also disrupts confluent monolayers of Madin-Darby canine kidney (MDCK) epithelial cells. DECMA-1 recognizes uvomorulin in extracts from mouse and dog tissues. Protease digestion of mouse and dog uvomorulin generated core fragments including one of 26 kd which reacted with DECMA-1. The same 26-kd fragment is recognized by anti-uvomorulin monoclonal antibodies which have been obtained from other laboratories and which dissociate MDCK cell monolayers and block the formation of the epithelial occluding barrier. This 26-kd fragment therefore seems to be involved in the adhesive function of uvomorulin.**
*Key words:* uvomorulin/cell adhesion/mouse embryo/MDCK cells

## Introduction

Our group is interested in the structure and function of the cell adhesion molecule (CAM) uvomorulin, which is involved in the $Ca^{2+}$-dependent compaction process of mouse morulae and the aggregation of embryonal carcinoma (EC) cells (Kemler *et al.*, 1977; Hyafil *et al.*, 1980). At later stages of embryonal development and in adult tissues, uvomorulin is exclusively expressed on epithelial cells, independent of their germ layer origin; on adult intestinal epithelial cells it is almost exclusively localized in the intermediate junctions (Boller *et al.*, 1985).

Uvomorulin is a 120-kd cell surface glycoprotein (gp120). Trypsin digestion in the presence of $Ca^{2+}$ releases an 84-kd fragment (UMt) (Hyafil *et al.*, 1980). Anti-gp120 and anti-gp84 antisera react with a set of proteins with mol. wts. of 120, 102 and 92 kd (Peyrières *et al.*, 1983; Vestweber and Kemler, 1984). The structure of uvomorulin and its tissue appearance are similar to chicken L-CAM (Gallin *et al.*, 1983) and human cell-CAM 120/80 (Damsky *et al.*, 1983). Another CAM, cadherin, appeared to be identical to uvomorulin (Yoshida-Noro *et al.*, 1984).

The adhesive function of CAMs have often been defined through polyclonal antibodies which disturb cell-cell contacts in a cell aggregation assay. Antibodies may act directly at a cell-adhesion site on CAMs, or indirectly via, for example, steric hindrance or induced conformational changes. The search for the functional cell adhesive site on CAMs is facilitated by the use of monoclonal antibodies reacting with just one epitope. Here we define more precisely a functional cell-adhesive site of uvomorulin with monoclonal antibodies directed against protease-resistant uvomorulin fragments. We show that the epitopes of three anti-uvomorulin monoclonal antibodies, each able to disturb cell-cell contact, are localized on a 26-kd fragment suggesting very strongly that this domain is involved in the mechanism of cell-cell adhesion.

## Results

### *A monoclonal antibody against uvomorulin decompacts mouse pre-implantation embryos and disrupts MDCK cells*

Rat monoclonal antibodies were first selected in an enzyme-linked immunoassay (ELISA) test against partially purified UMt followed by immunoprecipitation tests against iodinated UMt. Positive culture supernatants were tested for their ability to block aggregation of $PCC_4$aza1 cells. One hybridoma, DECMA-1 (decompacting monoclonal antibody), blocked the compaction of pre-implantation embryos (Figure 1, compare A and B). This antibody, an IgG1, was effective at 50-200 μg/ml when added to developing 8-cell embryos and had no visible effect on cell division. A control monoclonal antibody (ECMA-7; Kemler, 1980), which also reacts with the cell surface of pre-implantation embryos, had no effect on compaction. In the presence of DECMA-1, decompacted embryos recompacted after ~24 h and formed blastocysts even if the antibody solution was renewed (Figure 1C). Of 74 DECMA-1-treated embryos, 70 formed blastocysts which were composed of both trophectoderm and inner cell mass, as judged by light microscopy or monitoring with monoclonal antibody ECMA-3 (Kemler *et al.*, 1979). This antibody reacts on attached blastocysts specifically with the inner cell mass and is negative on trophectodermal cells (Figure 1D). Thus DECMA-1 acts differently to rabbit anti-uvomorulin Fab where no recompaction occurs in the presence of antibodies. An effect on cell aggregation was also observed when DECMA-1 was added to $PCC_4$aza1 cells both in tissue culture and in a $Ca^{2+}$-dependent cell aggregation assay (data not shown).

Since rabbit anti-uvomorulin antibodies react with adult epithelial cells, even from heterologous species (Boller *et al.*, 1985; Vestweber *et al.*, 1985), the functional effect of DECMA-1 on an established epithelial cell line was investigated. MDCK cells were cultured to confluency where the cells form a tight layer with poorly distinguishable cell boundaries (Figure 1E and G). The MDCK cells are polarized and form junctional complexes (Cereijido *et al.*, 1980). When DECMA-1 (20-500 μg/ml) or DECMA-1 Fab fragments (20-300 μg/ml) were added to these cells for 3-5 h they detached from each other and cell boundaries became easily recognizable (Figure 1, F and H). This effect started at the periphery of the confluent cell layer and progressed inwards. Cell-substratum interactions appeared not to be affected.

### *DECMA-1 binding studies*

*Immunoprecipitation and immunoblot experiments.* In immunoprecipitation experiments on cell lysates from EC cells, DECMA-1 recognized the same set of uvomorulin proteins (Figure 2, lane 5) of 120, 102 and 92 kd as rabbit anti-UM sera

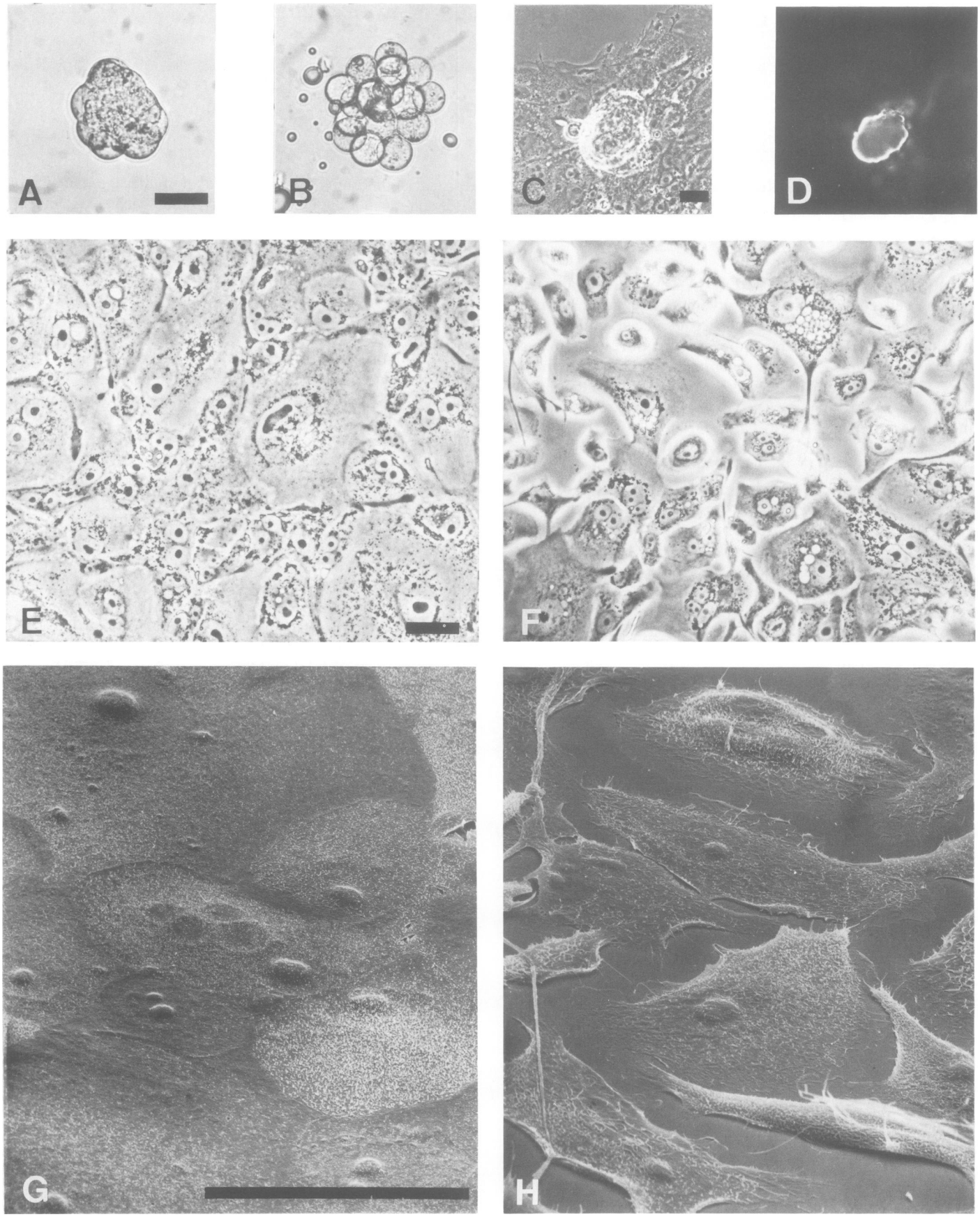


**Fig. 1.** Effect of monoclonal antibody DECMA-1 on cell-cell interaction. 8-cell embryos in Whitten's medium **(A)** or with the addition of 50 μg/ml DECMA-1 **(B)**, see Materials and methods. Recompacted embryos form blastocysts with trophectoderm and inner cell mass cells **(C)**. Indirect immunofluorescence staining of inner cell mass with monoclonal antibody ECMA-3 **(D)**. MDCK cells **(E – H)** incubated with 100 μg/ml of DECMA-1 for 5 h **(F** and **H)**, or with control antibodies **(E** and **G)**. Phase contrast **(E** and **F)** and scanning electron microscopy **(G** and **H)**. Bar represents: 50 μm.

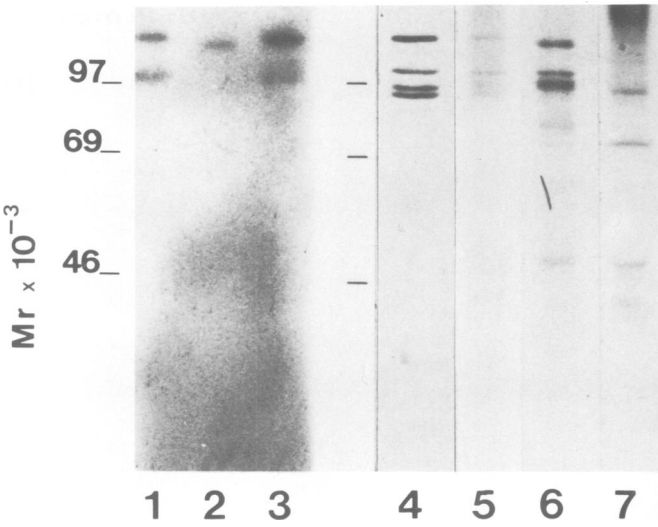


**Fig. 2.** Immunoblot and immunoprecipitation analysis with DECMA-1 and rabbit anti-uvomorulin on cell lysates of EC and MDCK cells. **Lanes 1–3** represent immunoblots of cell lysates of $PCC_4$aza1 **(1)**, MDCK **(2)** and F9 **(3)** cells using $^{125}$I-labelled DECMA-1 (sp. act. 25 $\mu$Ci/$\mu$g). Note that the 102-kd molecule is absent in MDCK cells. Immunoprecipitations **(4–7)** were carried out with cell lysates of [$^{35}$S]methionine-labelled $PCC_4$aza1 **(4,5,7)** and MDCK cells **(6)** using rabbit anti-UM **(4)**; rabbit non-immune **(7)** and DECMA-1 **(5** and **6)**.

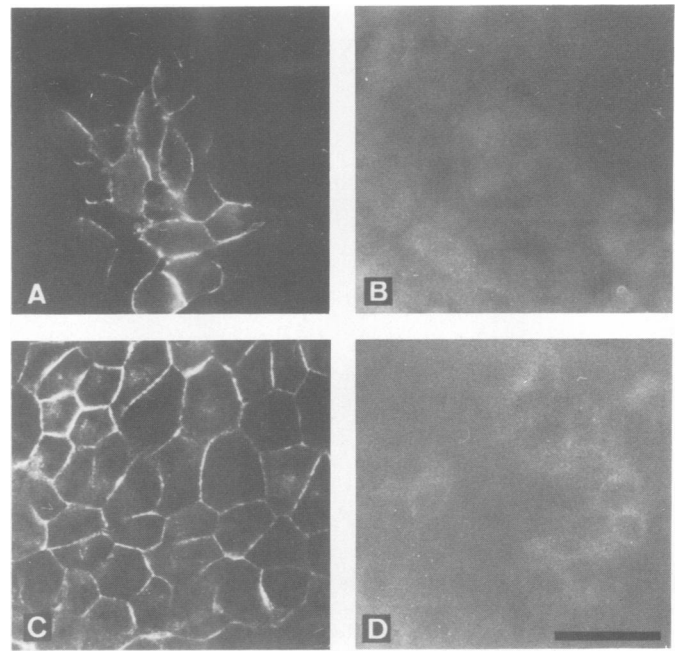


**Fig. 3.** Indirect immunofluorescence tests with DECMA-1 on $PCC_4$aza1 **(A,B)** and MDCK **(C,D)** cells grown on cover slips and fixed with 4% formaldehyde. DECMA-1 reacted with the cell surface of $PCC_4$aza1 cells **(A)** where labelling seemed to be concentrated on the membrane part of adjacent cells; control **(B)**. No staining was observed with DECMA-1 on formaldehyde-fixed MDCK cells **(D)** but when cells were subsequently treated with 1% Triton-X 100 a positive membrane staining with DECMA-1 was noted **(C)**. Bar represents 50 $\mu$m.

(Figure 2, lane 4) or monoclonal antibody DE-1 (Peyrièras *et al.*, 1983). The highest mol. wt. protein precipitated by DECMA-1 from cell lysates of MDCK cells migrated faster with an apparent mol. wt. of 115 kd (Figure 2, lane 6). This difference is not due to a different content of N-linked sugar moieties, as monitored by immunoprecipitations of EC and MDCK cells after tunicamycin treatment (1 $\mu$g/ml, 20 h). DECMA-1 recognized molecules of 113 kd instead of 120 kd from EC cells and 108 kd instead of 115 kd from MDCK cells (data not shown).

The 102-kd molecule is easily detectable in immunoprecipitation experiments but not in immunoblots (Figure 2, lane 2). This is due to SDS-sensitivity of antibody binding to the 102-kd molecule (Vestweber and Kemler, in preparation).

*Indirect immunofluorescence tests.* DECMA-1 labelled more strongly the cell-adjacent membrane than the peripheral part of the cell membrane of aggregated $PCC_4$aza1 cells following formaldehyde fixation (Figure 3A). However, a uniform ring of labelling was observed if tests were carried out with unfixed cells in suspension. With MDCK cells the antibody failed to stain the apical cell surface of unfixed or formaldehyde-fixed confluent monolayers (Figure 3D). However, if MDCK cells were treated with 1% Triton X-100 after formaldehyde fixation, strong membrane labelling occurred even in areas of confluency (Figure 3C).

*Radioactive assays.* Competitive binding studies with $^{125}$I-labelled and unlabelled DECMA-1 suggest that F9 and MDCK cells both express $\sim 2 \times 10^4$ DECMA-1 binding sites per cell (data not shown).

*Characterization of the DECMA-1 target on mouse uvomorulin*

The DECMA-1 target on UMt was characterized using the affinity column-purified 84-kd molecule labelled with $^{125}$I and digested with different proteases (see Materials and methods). Each digested sample was imunoprecipitated with rabbit anti-uvomorulin serum or DECMA-1 (Figure 4). From each enzyme digest, rabbit anti-uvomorulin serum precipitated discrete proteolytic fragments, while DECMA-1 precipitated only undigested material with one exception: after chymotrypsin (50 $\mu$g/ml) treatment in the presence of $Ca^{2+}$, DECMA-1 precipitated a protease-resistant fragment of 26 kd (Figure 4, lane 1). When $Ca^{2+}$ was omitted, but added for immunoprecipitations, DECMA-1 no longer reacted with any proteolytic fragment (Figure 4, lane 3). This was not due to complete proteolytic fragmentation by chymotrypsin since rabbit anti-uvomorulin serum still precipitated discrete proteolytic fragments under these conditions (Figure 4, lane 4). Separation of chymotrypsin/$Ca^{2+}$ digests on two-dimensional gels revealed the same isoelectric point of pH 4.5 for the 26-kd fragment as for UMt (data not shown). The 26-kd fragment is apparently free of methionine. When chymotrypsin/$Ca^{2+}$ digests of [$^{35}$S]methionine and $^{125}$I-labelled cells were subjected to immunoprecipitations with DECMA-1, the 26-kd fragment was only seen from digests of iodinated cells (data not shown).

*The targets of three 'functional' monoclonal antibodies are also localized on the 26-kd fragment of dog uvomorulin*

Two monoclonal antibodies, anti-Arc-1 and rr1 have been described with similar effects to DECMA-1 on MDCK monolayers. While anti-Arc-1 was selected for its ability to disrupt MDCK monolayers (Imhof *et al.*, 1983), the rr1 antibody selectively blocked the reformation of the occluding barrier of the junctional complexes in MDCK monolayers (Gumbiner and Simons, 1985). With rabbit anti-uvomorulin antibodies and DECMA-1 it was shown that both anti-Arc-1 and rr1 recognize the canine uvomorulin (Behrens *et al.*, 1985; Gumbiner and Simons, 1985). Thus, three monoclonal antibodies directed against uvomorulin are available that disturb the cell-cell contacts of MDCK cells. We attempted to localize the anti-Arc-1 and rr1 targets on uvomorulin as described above for DECMA-1. Since anti-Arc-1 and rr1 are mouse antibodies and do not react with mouse

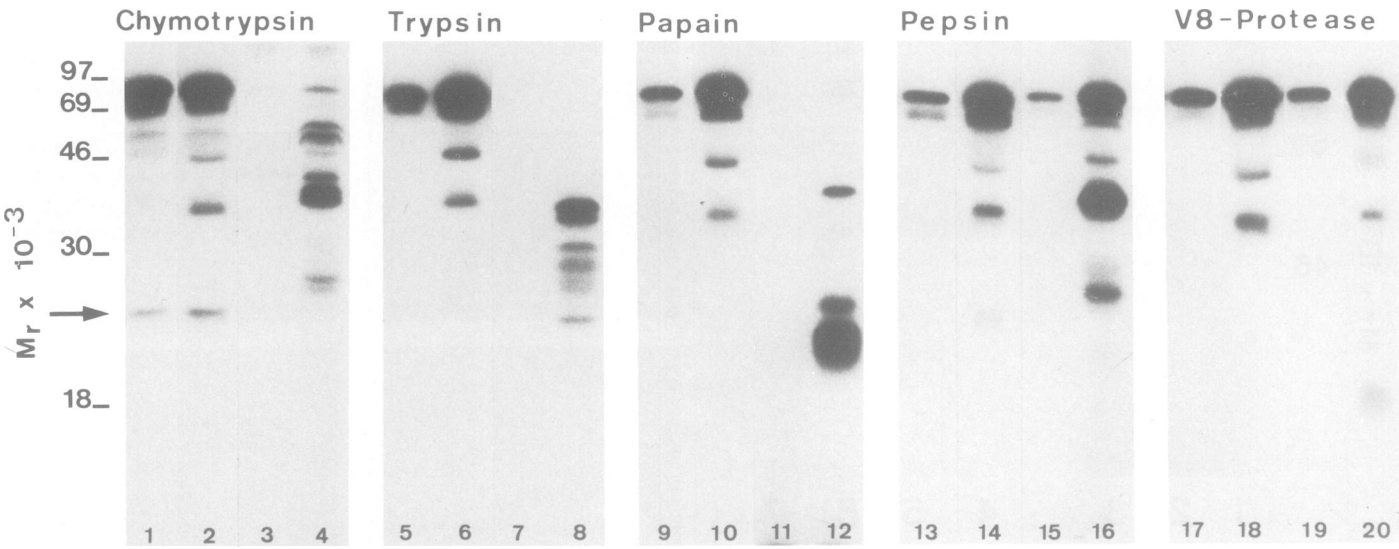


**Fig. 4.** Immunoprecipitations of mouse UMt treated with different proteases. Affinity-purified $^{125}$I-labelled UMt was digested with chymotrypsin (50 μg/ml); trypsin (10 μg/ml); papain (10 μg/ml); pepsin (50 μg/ml) and V8-protease (10 μg/ml) as described in Materials and methods in the presence **(lanes 1,2,5,6,9,10,13,14,17,18)** or absence **(lanes 3,4,7,8,11,12,15,16,19,20)** of $Ca^{2+}$. Digested material was immunoprecipitated with DECMA-1 (odd lanes) and rabbit anti-UM (even lanes). DECMA-1 precipitated a peptide fragment of 26 kd only after chymotrypsin digestion in the presence of $Ca^{2+}$ **(lane 1)** while with rabbit anti-UM serum fragments with different sizes were precipitated. Immunocomplexes were separated by SDS-PAGE and autoradiographs were exposed for 1 day at −70°C. Arrow indicates the position of the 26-kd fragment.

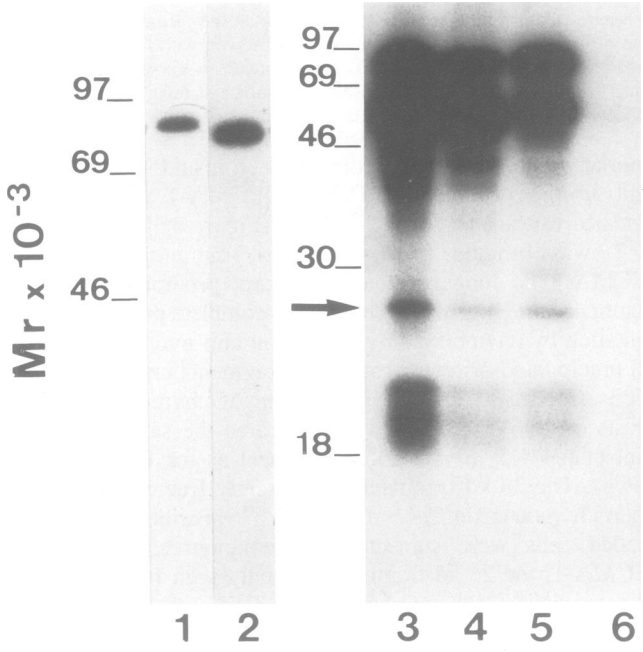


**Fig. 5.** Immunoprecipitations with DECMA-1, anti-Arc-1 and rr1 of mouse and dog UMt and protease-digested dog UMt. **Lanes 1** and **2**: affinity column-purified and $^{125}$I-labelled mouse **(1)** and dog **(2)** UMt precipitated with DECMA-1. Dog UMt migrates at a mol. wt. of ~80 kd, mouse UMt at 84 kd. **Lanes 3–6**: $^{125}$I-labelled dog UMt was digested with 1 mg/ml chymotrypsin in the presence of $Ca^{2+}$ and digests were immunoprecipitated with DECMA-1 **(3)**, anti-Arc-1 **(4)**, rr1 **(5)** and a mouse IgG1 monoclonal antibody with an unrelated specificity **(6)**. DECMA-1, anti-Arc-1 and rr1 precipitated an identical pattern of proteolytic fragments with mol. wts. of 26, 20 and 19 kd. Immunocomplexes were separated by 10% **(1** and **2)** and 15% **(3–6)** SDS-PAGE and autoradiographs were exposed for 2 days at −70°C. Arrow indicates the position of the 26-kd proteolytic fragment.

uvomorulin, these experiments were carried out on dog UMt isolated from kidney. When immunoprecipitates of iodinated mouse and dog UMt were analyzed on SDS-PAGE the dog UMt migrated slightly faster (mol. wt. ~80 kd) than the 84-kd mouse UMt (Figure 5, lanes 1 and 2). When dog UMt was digested with chymotrypsin/$Ca^{2+}$ as described above for mouse UMt the yield of proteolytic fragments was approximately five times lower (as assessed by the intensity of the precipitated 26-kd fragment). The three monoclonal antibodies recognized the proteolytic fragment of 26 kd from samples digested with 50 μg/ml chymotrypsin. When higher chymotrypsin concentrations (>1mg/ml) were used all antibodies precipitated an identical pattern of proteolytic fragments with mol. wts. of 26, 20 and 19 kd, although other minor fragments could be seen in variable amounts (Figure 5, lanes 3–5). From these experiments we conclude that the targets of the three monoclonal antibodies are located on the same proteolytic fragments of dog uvomorulin.

## Discussion

Uvomorulin is a major cell adhesion molecule (CAM) in several mammalian species and may play an important physiological role during embryonic development and in adult epithelia. Consonant with this we have shown here that antibody DECMA-1 decompacts mouse pre-implantation embryos and dissociates monolayers of canine MDCK epithelial cells.

Monoclonal antibodies reacting with mouse uvomorulin have been described. DE1 selected by Hyafil and co-workers (1981) against UMt has no effect on the aggregation of EC cells or the compaction of pre-implantation embryos, but it binds only in the presence of $CA^{2+}$ which suggests that uvomorulin undergoes a $Ca^{2+}$-dependent conformational change. Binding of DECMA-1 is also $Ca^{2+}$-dependent. Our studies underline the importance of $Ca^{2+}$ for the conformation of uvomorulin. $Ca^{2+}$ promotes the resistance of UMt not only to trypsin but also to several other proteases. What we show here is that this protective effect acts on a region of the molecule necessary for cell adhesion activity, as defined by three monoclonal antibodies.

The monoclonal antibody ECCD-1 described by Yoshida-Noro *et al.* (1984) is most similar to DECMA-1 with respect to the cell-cell interaction effect on different cell types. The ECCD-1 target was termed cadherin, but subsequent analysis showed that

cadherin is identical to uvomorulin (Yoshida-Noro *et al.*, 1984). ECCD-1, like DECMA-1, interferes with the $Ca^{2+}$-dependent cell-cell adhesion of teratocarcinoma cells, the compaction of mouse pre-implantation embryos and affects cell-cell adhesion of epithelial cells. Neither ECCD-1 nor DECMA-1 totally inhibit the compaction process of pre-implantation mouse embryos: the embryo can overcome the effect after a delay of ~24 h. There is, however, one important difference between these two antibodies. Shirayoshi *et al.* (1983) showed that blastocysts grown in the presence of ECCD-1 do not form inner cell mass cells. We do not find such an effect on the inner cell mass formation with DECMA-1.

Our study establishes that uvomorulin is an adhesive factor for MDCK cells. Our immunofluorescence data show that antibody binding to uvomorulin on highly confluent MDCK cells could only be detected after detergent treatment of the fixed cell layers which presumably allows the antibodies to by-pass the tight junctional barrier. This is consistent with data from electron microscopic studies on mouse and rat intestine where no antibody binding to the apical part of the epithelial cells could be detected (Boller *et al.*, 1985). The immunofluorescence results in which DECMA-1 stained unpermeabilized MDCK cells only in the periphery of the confluent cell layer are consistent with the observation that DECMA-1 first begins to dissociate MDCK cells at the periphery of the confluent cell layer, and progressively disrupts cell-cell adhesion in confluent areas.

Two other monoclonal antibodies, anti-Arc-1 and rr1 have a similar effect to DECMA-1 on the cell-cell contact of MDCK cells. Anti-Arc-1 was obtained from mice immunized with MDCK cells and was selected by its ability to dissociate MDCK monolayers (Imhof *et al.*, 1983). Antibody rr1 was selected in a resistance recovery assay where the antibody blocked the reformation of the junctional complexes in MDCK monolayers (Gumbiner and Simons, 1985). Although the selection criteria for both antibodies were different to DECMA-1, subsequent comparison with anti-uvomorulin antibodies and DECMA-1 showed that both, anti-Arc-1 and rr1, recognize uvomorulin on MDCK cells (Behrens *et al.*, 1985; Gumbiner and Simons, 1985). This provided us with the opportunity to ask if these antibodies selected by different functional criteria recognize the same or different epitopes on the uvomorulin molecule. Different uvomorulin protease-resistant domains were generated and the DECMA-1 target was localized on a 26-kd fragment of uvomorulin obtained after chymotrypsin digestion. Revealingly, this 26-kd fragment also harbours the antigenic targets of antibody anti-Arc-1 and rr1. More extensive chymotrypsin digestion may give rise to smaller fragments (e.g., at 19 and 20 kd) in rather variable amounts. Since the epitope-bearing 26-kd fragment is generated in relatively high yield under mild conditions it would seem to be a good starting point for further structural studies. Our results suggest that the 26-kd uvomorulin domain is involved in the molecular mechanism of cell adhesion. It is noteworthy, in this respect, that the binding of other proteins to uvomorulin does not effect cell adhesion: succinyl ConA (Kemler *et al.*, 1977) as well as monoclonal antibody DE-1 (Hyafil *et al.*, 1981) both bind to uvomorulin but do not interfere with cell adhesion. The DECMA-1, anti-Arc-1 and rr1 epitopes map together as closely as 19 kd on the 120-kd uvomorulin molecule. The simplest explanation for these data is that this uvomorulin region bears at least one adhesive site. It will be necessary to determine whether the 26-kd fragment interferes with cell adhesion or whether it inhibits binding of uvomorulin to cells. The 26-kd fragment apparently contains no methionine. This fact could be helpful in localizing this presumptive uvomorulin cell adhesion domain within the uvomorulin molecule once DNA sequencing data are available from the recently isolated uvomorulin cDNA (Schuh *et al.*, 1986).

In conclusion, we have found that three monoclonal antibodies that affect cell-cell interactions, all interact with a 26-kd fragment of uvomorulin. This suggests that a unique immunogenic cell-interaction domain exists whose precise molecular structure we can now define.

## Materials and methods

*Cell culture*

All cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 15% fetal calf serum (FCS) in a 10% $CO_2$ atmosphere. $PCC_4$aza1 (Nicholas *et al.*, 1975) and F9 (Bernstine *et al.*, 1973) are EC cell lines and MDCK is a canine kidney epithelial cell line (ATCC, 1981). Where large quantities of F9 cells were needed, cells were cultured for 72 h in suspension culture under rotary shaking (120 r.p.m.) in DMEM, 15% of FCS and 20 mM Hepes. Mouse pre-implantation embryos were obtained from superovulated 129/Sv or C57BL/6 females and cultured under paraffin oil in Whitten's medium as described earlier (Kemler *et al.*, 1977).

*Hybridoma production*

Lou rats were immunized with $PCC_4$aza1 cells. Each animal received $10^7$ cells i.p. at 2-week intervals. For the first immunization, cells were mixed with complete Freund's adjuvant (v/v), all subsequent injections were done in phosphate-buffered saline. After the third immunization the sera were tested for decompaction activity on $PCC_4$aza1 cells (Vestweber and Kemler, 1984). Animals with positive titers each received i.v. on two subsequent days 50 μg of a partially purified UMt fraction (see below) and spleens were removed 3 days after the last injection. Spleen cells were fused with rat myeloma cells, Y3-Ag1.2.3. (Galfre *et al.*, 1979) as previously described (Kemler *et al.*, 1979). Hybrid growth was selected in HAT medium (Köhler and Milstein, 1975).

Culture supernatants from hybrid cells were screened in an ELISA test against the UMt protein fraction and positive supernatants were re-examined by immunoprecipitation tests against the iodinated UMt protein fraction. Only culture supernatants which recognized an 84-kd protein were kept for further investigations. Hybrid cells were subcloned twice by single cell cloning with mouse peritoneal macrophages as feeder cells. The secreted monoclonal antibody was characterized in an Ouchterlony test using anti-subclass specific antisera (Nordic). Ascites fluid was obtained by injecting ~$10^7$ hybridoma cells i.p. in Pristan (Roth, Karlsruhe) treated Lou rats.

*Antisera*

The production of rabbit anti-uvomorulin sera and their monovalent Fab fragments has been described previously (Vestweber and Kemler, 1984). Monovalent Fab fragments of DECMA-1 were produced according to the same procedure. Affinity column-purified rabbit anti-rat IgG antibodies were isolated from hyperimmunized animals by passing the antisera over a CNBr-Sepharose 4B (Pharmacia) column coupled with rat IgG (Miles). Sheep $F(ab)_2$ anti-rat IgG peroxidase-labelled or FITC-conjugated were obtained from Cappel.

*Purification of mouse and dog UMt*

Partial purification of mouse UMt was done by trypsinizing (Hyafil *et al.*, 1980) plasma membranes of F9 cells (Vestweber and Kemler, 1984). Proteins eluted from a Concanavalin A-Sepharose column were subsequently fractionated by DEAE-Sepharose chromatography and protein fractions were examined for the presence of gp84 by means of immunoblot experiments. The partially purified UMt fraction contained ~40% of gp84 as judged by silver staining (Oakley *et al.*, 1980) of SDS-PAGE (Laemmli, 1970) separated proteins.

Affinity-purified UMt was prepared using a DECMA-1 Sepharose column (5 mg protein/ml gel matrix). Mouse UMt was purified from trypsinized plasma membranes of F9 cells and dog UMt was prepared from kidney. For this, one dog kidney in 200 ml of 2 mM $CaCl_2$ was homogenized in a Waring blender for 1 min. The homogenate was filtered through a coarse sieve, centrifuged at 7000 *g* and the pellet was trypsinized with 0.2 mg/ml trypsin (Sigma, type XI) in 25 ml of 10 mM Tris-HCl, pH 7.4; 100 mM NaCl; 2 mM $CaCl_2$. Trypsinization was stopped by adding 10 mg soybean trypsin inhibitor. The trypsin extract was cleared (10 000 *g*, 30 min) and passed over the DECMA-1 affinity column. The eluted UMt fraction was free of contaminating proteins as judged by silver staining of SDS-PAGE separated proteins.

*Proteolytic cleavage of UMt*

Enzyme digestions of affinity column-purified UMt was performed on $^{125}$I-labelled gp84. For this, 10 μg of gp84 were iodinated with 1 mCi Na-$^{125}$I (Amersham)

using iodogen (Pierce) as oxidizing reagent (Fraker and Speck, 1978). Free iodine was removed on a Sephadex G-25 column. The following proteases were used in various concentrations: *Staphylococcus* V8 (Miles), 10 μg/ml in 125 mM Tris-HCl, pH 6.8; papain (Sigma, type III), 5, 10 and 50 μg/ml in 10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 10 mM cysteine; pepsin (Sigma) 5 and 50 μg/ml in 100 mM Na-acetate pH 4.0; trypsin (Sigma, type XI), 1 and 10 μg/ml; and chymotrypsin A4 (Boehringer) 0.01 – 1 mg/ml in 10 mM Tris-HCl, pH 7.4, 100 mM NaCl. Enzyme digestion was done by adding 10 μl containing 0.3 μg [$^{125}$I]gp84 (16 μCi/μg) to 60 μl enzyme solution for 30 min at 37°C either in the presence or absence of 1 mM $CaCl_2$. The digestion was stopped on ice by adding 3 mM phenylmethylsulfonyl fluoride (PMSF) to V8 protease, trypsin or chymotrypsin; 0.5 μg/ml antipain to papain; and by changing the pH to 8.0 in the case of pepsin. Each digested sample was immunoprecipitated with anti-UM, pre-immune serum and monoclonal antibodies.

*Radioactive labelling and immunoprecipitation*

$PCC_4$aza1 and MDCK cells were labelled for 16 h with [$^{35}$S]methionine (Amersham, sp. act. 1100 μCi/mmol) 20 μCi/ml DMEM, without methionine and supplemented with 10% FCS. Freshly harvested cells were labelled with $^{125}$I using the lactoperoxidase technique (Vestweber and Kemler, 1984). Cells were washed three times in PBS to remove free radioactivity and either lysed with 1% Triton-X 100 (Vestweber and Kemler, 1984), or treated with chymotrypsin (0.1 mg/ml chymotrypsin, Boehringer) in Hepes-buffered saline, 2 mM $CaCl_2$ for 60 min at 37°C. Enzymatic digestion was stopped by adding 0.1 mg/ml soybean trypsin inhibitor and insoluble material was removed by ultracentrifugation at 100 000 *g* for 45 min.

Immunoprecipitations were carried out with fixed *Staphylococcus* Cowan strain I (Staph A) (Kessler, 1975) as described earlier (Vestweber and Kemler, 1984). Briefly, 2 – 3 × $10^6$ TCA-precipitable c.p.m. of labelled cell extracts or 5 × $10^5$ c.p.m. of [$^{125}$I]UMt were incubated with 200 μl Staph A to remove unspecific binding. The Staph A were pelleted and the supernatants were incubated with 20 μl of rabbit antiserum or 20 μg of monoclonal antibody for 45 min at 4°C. In the case of monoclonal antibodies, 10 μg of purified rabbit anti-rat-IgG were added as intermediate reagent. Immunocomplexes were precipitated with 100 μl Staph A, washed and proteins were separated on 1- or 2-dimensional polyacrylamide gels (Laemmli, 1970; O'Farrell, 1975) and subsequently analyzed by autoradiography.

*Immunoblot*

Experiments were carried out as described (Vestweber and Kemler, 1984) with the exception that Hb/PBS (PBS, 2% hemoglobin, 0.05% Tween 20) was used for all washings and antibody dilutions.

*Radioactive binding assays*

Tests were done as described (Behrens *et al.*, 1985) using affinity column-purified DECMA-1 $^{125}$I-labelled with iodogen (Fraker and Speck, 1978) at 25 μCi/μg protein.

*Indirect immunofluorescence tests*

Cells grown on cover slips were rinsed in PBS and fixed for 10 min in freshly prepared 4% formaldehyde in PBS. After several washes in PBS, formaldehyde-fixed cells were treated in some cases for 15 min with 1% Triton-X 100 in PBS and washed again in PBS. Indirect immunofluorescence tests were done in a humid chamber as described (Vestweber and Kemler, 1984). Monoclonal antibody binding (10 – 50 μg/ml) was detected with rabbit $F(ab)_2$ anti-rat IgG-FITC (Cappel), or in a triple sandwich test with rabbit anti-rat IgG (10 μg/ml, affinity column purified) as an intermediate reagent. Stained specimens were mounted in glycerol/PBS (80:20 v/v) and were observed in a Leitz-Dialux E20 fluorescence microscope. Photographs were taken using Ilford HP-5 film.

*Adhesion assays*

*Decompaction of pre-implantation embryos.* Zona pellucida-free 8-cell embryos were cultured in Whitten's medium (Kemler *et al.*, 1977) and monoclonal antibodies were tested for decompaction activity at concentrations of 50 – 100 μg/ml.

*Aggregation assay.* The antibody effect on aggregating $PCC_4$aza1 cells was studied in a cell aggregation assay (Vestweber *et al.*, 1985).

*Decompaction of cell monolayers.* Decompaction was tested on $PCC_4$aza1 and MDCK cells cultured in DMEM supplemented with 10% heat-inactivated FCS. $PCC_4$aza1 cells were cultured in microtest tissue culture plates (Costar) at 1 – 2 × $10^4$ cells per well. MDCK cells were plated at different densities on glass cover slips. Cells were incubated for 4 – 5 h with anti-uvomorulin serum (1/50 and 1/500 dilutions), their Fab fragments (100 and 200 μg/ml), monoclonal antibodies (10, 50, 100, 200 and 1000 μg/ml) or Fab of monoclonal antibodies (50, 100, 200 and 500 μg/ml). Controls were included using pre-immune serum and Fab fragments as well as monoclonal antibody ECMA-7 (Kemler, 1980).

The antibody effect was observed by light microscopy or by scanning electron microscopy. For scanning electron microscopy cells were fixed in 2.5% glutaraldehyde in PBS for 3 h at room temperature, dehydrated and critical-point-dried from $CO_2$. The specimens were sputter-coated with 20 nm platinum and inspected in an ETEC scanning electron microscope.

## Acknowledgements

We thank Mr. Werner Schmidt, Ms. Cordula Müller, Ms. Christa Baradoy and Ms. Margot Katz for excellent technical assistance; and J. Behrens (anti-Arc-1) and Dr. B. Gumbiner (rr1) for the generous gift of their monoclonal antibodies. We also acknowledge the help and advice of Dr. H. Frank and Mr. J. Sauter in preparing the raster electron micrographs. We thank Drs. S. Goodman and D.F. Newgreen for critically reading and R. Brodbeck for typing the manuscript.

## References

ATCC (1981) *American Type Culture Collection: Catalogue of Strains II*, ATCC, Rockville, MD, p. 52.

Behrens,J., Birchmeier,W., Goodman,S.L. and Imhof,B.A. (1985) *J. Cell. Biol.*, in press.

Bernstine,E.P., Hooper,M.L., Grandchamp,S. and Ephrussi,B. (1973) *Proc. Natl. Acad. Sci. USA*, **70**, 3899-3902.

Boller,K., Vestweber,D. and Kemler,R. (1985) *J. Cell Biol.*, **100**, 327-332.

Cereijido,M., Ehrenfeld,J., Meza,I. and Martinez-Palomo,A. (1980) *J. Membr. Biol.*, **52**, 147-159.

Damsky,C.H., Richa,J., Solter,D., Knudsen,K. and Buck,C.A. (1983) *Cell*, **34**, 455-466.

Fraker,P.J. and Speck,J.C. (1978) *Biochem. Biophys. Res. Commun.*, **80**, 849-857.

Gallin,W.J., Edelman,G.M. and Cunningham,B.A. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 1038-1042.

Galfre,G., Milstein,C. and WRight,B. (1979) *Nature*, **277**, 131-133.

Gumbiner,B. and Simons,K. (1985) *J. Cell Biol.*, in press.

Hyafil,F., Morello,D., Babinet,C. and Jacob,F. (1980) *Cell*, **21**, 927-934.

Hyafil,F., Babinet,C. and Jacob,F. (1981) *Cell*, **26**, 447-454.

Imhof,B.A., Volmers,H.P., Goodman,S.L. and Birchmeier,W. (1983) *Cell*, **35**, 667-675.

Kemler,R. (1980) in Sauer,H.W. (ed.), *Progress in Developmental Biology*, Gustav Fischer Verlag, Stuttgart, pp. 175-181.

Kemler,R., Babinet,C., Eisen,H. and Jacob,F. (1977) *Proc. Natl. Acad. Sci. USA*, **79**, 4449-4452.

Kemler,R., Morello,D. and Jacob,F. (1979) in Le Douarin,N. (ed.), *Cell Lineage, Stem Cells and Cell Determination, INSERM Symposium No. 10*, Elsevier/North Holland Biomedical Press, pp. 101-113.

Kessler,S.W. (1975) *J. Immunol.*, **115**, 1617-1624.

Köhler,G. and Milstein,C. (1975) *Nature*, **256**, 495-497.

Laemmli,U.K. (1970) *Nature*, **227**, 680-685.

Nicolas,J.F., Dubois,P., Jakob,H., Gaillard,J. and Jacob,F. (1975) *Ann. Microbiol. (Inst. Pasteur)*, **126A**, 3-22.

Oakley,B.R., Kirsch,D.R. and Morris,N.R. (1980) *Anal. Biochem.*, **105**, 361-363.

O'Farrell,P.M. (1975) *J. Biol. Chem.*, **250**, 4007-4021.

Peyrières,N., Hyafil,F., Louvard,D., Ploegh,H. and Jacob,F. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 6274-6277.

Schuh,R., Vestweber,D., Riede,J., Rosenberg,U., Jäckle,H. and Kemler,R. (1986) *Proc. Natl. Acad. Sci. USA*, in press.

Shirayoshi,Y., Okada,T.S. and Takeichi,M. (1983) *Cell*, **35**, 631-638.

Vestweber,D. and Kemler,R. (1984) *Exp. Cell. Res.*, **152**, 169-178.

Vestweber,D., Ocklind,C., Gossler,A., Odin,P., Öbrink,B. and Kemler,R. (1985) *Exp. Cell. Res.*, **137**, 451-461.

Yoshida-Noro,C., Suzuki,N. and Takeichi,M. (1984) *Dev. Biol.*, **101**, 19-27.

*Received on 20 September 1985*