DISTRIBUTION OF DESMOSOMAL COMPONENTS IN THE TISSUES OF VERTEBRATES, STUDIED BY FLUORESCENT ANTIBODY STAINING

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SUMMARY

In previous work we used immunofluorescent staining with specific antibodies to study the distribution of five desmosomal antigens in the epithelia of different vertebrate animals. We showed that all five antigens were present in all epithelia studied in human, bovine, rat, guinea pig, chick and frog (Rana pipiens) tissues. It was concluded that desmosomes are highly conserved structures.

This paper extends those studies: (a) by including three other species, a lizard (Lacerta viridis), the axolotl (Ambystoma mexicanum) and the trout (Salmo trutta), and (b) by looking at several tissues in more detail.

The principal results are as follows. (1) The epidermis of all species down to the frog stain with equal intensity for all desmosomal antigens. (2) In the epidermis of axolotl and trout, staining for desmosomal plaque constituents is present, but staining for the desmosomal glycoproteins is greatly reduced or absent. (3) Within mammalian species as well as chick, lizard and frog, staining for the 115 × 10^3 and 100 × 10^3 molecular weight desmosomal glycoproteins is less intense in non-epidermal tissues than in the epidermis, while staining for desmosomal plaque constituents and for the 150 × 10^3 molecular weight glycoprotein is undiminished. It is possible, therefore, that slight differences exist between certain glycoproteins of epidermis and non-epidermal epithelia. (4) The hearts of lower vertebrates (lizard, frog, axolotl and trout) stain only for individual desmosomal plaque antigens. (5) The pillar cells of trout gill stain, adjacent to their collagenous columns, with one desmosomal plaque antigen. There is a fibrous cytoplasmic mat in this position but no desmosomes. Thus one of the desmosomal antigens may have a function outside the desmosome.

INTRODUCTION

Desmosomes or maculae adhaerentes (Farquhar & Palade, 1963) are cell membrane organelles involved in intercellular adhesion (Overton, 1975). They occur in most epithelia but are also present in the intercalated discs of vertebrate heart muscle. They consist of parallel cell membranes separated by an intercellular space of 25–35 nm. This space contains extracellular material, characteristically organized into an electron-dense mid-line halfway between the opposed plasma membranes. On the cytoplasmic side of the membranes are dense, rigid plaques that are 10–15 nm in thickness and may be separated from the inner leaflet of the membrane by a 3 nm gap. Connected to the plaques are cytoplasmic tonofilaments of cytokeratin (Farquhar & Palade, 1963; Kelly, 1966; McNutt & Weinstein, 1973; Staehelin, 1974; Overton, 1974; Henderson & Weber, 1981).

The isolation of desmosomes from bovine nasal epithelium was achieved by Skerrow & Matoltsy (1974). More recently, Gorbsky & Steinberg (1981) have adapted the
isolation procedure to obtain desmosomal cores, structures consisting of the membrane and intercellular components but with greatly reduced plaques and devoid of tonofilaments. Desmosomal cores consist of five major groups of proteins as follows: a glycoprotein of $22 \times 10^3 M_r$, two proteins of $82 \times 10^3$ and $86 \times 10^3 M_r$, a glycoprotein of $100 \times 10^3 M_r$ and a glycoprotein doublet of $115 \times 10^3 M_r$, a glycoprotein triplet of $150 \times 10^3 M_r$, and two proteins of $205 \times 10^3$ and $230 \times 10^3 M_r$. The glycoproteins are enriched in cores and are therefore thought to be intercellularly located, while the proteins are thought to be plaque constituents (Gorbsky & Steinberg, 1981). The plaque location of the $230 \times 10^3$ and $205 \times 10^3 M_r$ proteins has been supported by the work of Franke et al. (1982).

We have raised polyclonal antisera against each of the five high molecular weight desmosomal components (i.e. every protein except the $22 \times 10^3 M_r$) (Cowin & Garrod, 1983). Having determined the specificity of these antisera by immunoblotting, we showed that each gave a staining pattern corresponding to the distribution of desmosomes in a range of tissues from different vertebrate species (man, cow, rat, guinea pig, chick and frog (Rana pipiens)). We concluded that desmosomal components are highly conserved, as has been previously suggested from studies showing that desmosomes from different vertebrate sources have similar ultrastructure (reviewed by Overton, 1975). (Similar organelles also occur in invertebrates, but their ultrastructure is more variable.)

The purpose of the present paper is to extend our survey of desmosomes in vertebrate tissues. We have included a lizard (Lacerta viridis), the axolotl (Ambystoma mexicanum) and the trout (Salmo trutta). Our results suggest several important conclusions. Firstly, the epidermal desmosomes of all species from man to frog are indistinguishable with regard to staining for any of the desmosomal antigens. In the axolotl and trout, however, staining for the desmosomal glycoproteins is lost. Secondly, in the non-epidermal tissues of higher vertebrates (man to lizard) staining for the $115 \times 10^3$ and $100 \times 10^3 M_r$ glycoproteins is diminished in intensity, whereas that for plaque components and the $150 \times 10^3 M_r$ glycoprotein is as intense as in the epidermis. This may mean that certain glycoproteins of non-epidermal desmosomes differ somewhat from those of the epidermis. Thirdly, one desmosomal component, the $82 \times 10^3 M_r$ antigen or a related protein, seems to have an extra-desmosomal location in fish gills.

**MATERIALS AND METHODS**

**Methods**

Our methods for isolation of desmosomes and desmosomal antigens, for immunization of guinea pigs, for immunoblotting and for fluorescent antibody staining have been described previously (Cowin & Garrod, 1983). In this paper intensity of fluorescent antibody staining has been classified on an arbitrary seven-point scale as follows: —, staining absent; +/—, staining equivocal; 1+, 2+, 3+, 4+, 5+, gradually increasing scale of brightness from just detectable to very bright. With practice it became possible to identify these scale points so that independent observers agreed within one point.
**Distribution of desmosomal components**

**Electron microscopy**

Blocks of tissues (2 mm) were washed three times in PBS and fixed in 2.5% glutaraldehyde in sodium cacodylate at pH 7.2 for 1 h. They were washed with three changes of cacodylate buffer containing 7.5% sucrose, post-fixed in 1% osmium tetroxide for 1 h, washed three times in distilled water and stained en bloc with 1% uranyl acetate for 30 min. After washing three times with distilled water the tissues were dehydrated through an ethanol series and embedded in Spurr resin.

**RESULTS**

**Specificity of antibodies**

The specificity of our antibodies as determined by immunoblotting has been discussed previously (Cowin & Garrod, 1983). Here it is necessary to mention that two of the antisera raised against the $86 \times 10^3M_r$ protein reacted with both the $86 \times 10^3$ and $82 \times 10^3M_r$ proteins, while a third reacted with the $86 \times 10^3M_r$ protein only. These different antisera had different staining properties as shown below.

**Epidermis**

Essentially similar patterns of staining to that described for bovine nasal epithelium (Cowin & Garrod, 1983) were obtained with the skins of all species. (A section of bovine nasal epithelium is shown in Fig. 1.) In the frog, however, staining of the dermal–epidermal junction was found (Fig. 2). Human skin showed a reduction in staining intensity in the stratum corneum (Fig. 3). In contrast, cornea (chick embryo) showed a pattern of staining consistent with the distribution of desmosomes in a non-keratinizing epithelium, staining intensity increasing towards the outer surface (Fig. 4). As shown in Table 1 staining of the epidermis occurred equally intensely with all antibodies in all species, with the following exceptions. Axolotl showed slightly reduced staining for $86 \times 10^3$ and $82 \times 10^3$, very weak staining for $150 \times 10^3$ and no staining for $115 \times 10^3$ and $100 \times 10^3M_r$ proteins. Staining for all three glycoproteins was absent in fish.

**Liver**

Punctate staining was obtained with all antibodies and corresponded with the

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Table 1. Staining of epidermis from a range of species with antibodies to desmosomal components
The occurrence of desmosomal antigen staining in the livers of all species is shown in Table 2. Reaction for the plaque proteins was quite distinct in each case. In
Distribution of desmosomal components

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Contrast, staining with anti-115 and anti-100 was much less bright than in the epidermis but nevertheless was present in all species down to and including the lizard. In the frog, staining with these antibodies was equivocal, and in axolotl and fish it was completely absent. Staining for the $150 \times 10^3 M_r$ antigen was intense in all species down to the frog, just detectable in axolotl but absent in trout.

Gut

The data presented for the gut have been pooled from those obtained for stomach and small intestine, each of which gave similar results. The distribution of epithelial staining was punctate and more localized than in epidermis, being particularly concentrated in the terminal bar regions between the lateral surfaces of the epithelial cells (Figs 8, 9, 10). No staining was found in non-epithelial cells of these tissues.

Staining of gut showed the same occurrence of antigens as liver except that staining with anti-100 was absent in the lizard (Table 3).

Heart

Staining of the heart ventricle, unlike that of other tissues, presented a variety of patterns in different species.

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**Fig. 1.** Section of bovine snout including regions of dermis (d) and epidermis, showing staining of the epidermal cell boundaries with anti-100. Note the outlines of the prickle-like projections of these cells (arrows) and the absence of staining of the dermal–epidermal junction (arrowheads). Bar, 20 μm. (From Cowin & Garrod (1983) with kind permission from Macmillan Journals Ltd.)

**Fig. 2.** Section of frog skin stained with anti-205/230. Note the punctate pattern of staining and the staining of regions of the dermal–epidermal junction (arrowhead). Bar, 20 μm (in Figs 1–18).

**Fig. 3.** Section of human skin stained with anti-150 showing absence of staining of the dermis (d) and dermal–epidermal junction. Staining intensity is greatest around the prickle cells of the stratum spinosum (s) and decreases in the outer layers of the stratum corneum (c) reflecting the gradual deterioration of the desmosomes in the outer layers, which ultimately leads to the sloughing of the skin cells.

**Fig. 4.** Section of embryonic chick cornea stained with anti-100 showing the greater intensity of staining in the outer layers, which reflect the typical distribution of desmosomes in non-keratinizing epidermis.
Figs 5-10
Table 3. Staining of gut from a range of species with antibodies to desmosomal components

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<tr>
<th>Antibody</th>
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Gut, oesophagus, stomach and intestine.

All antisera stained the intercalated discs of mammalian heart (Figs 11, 12). In chick, all antisera stained the intercalated discs together with punctate staining along and between the muscle fibres (Fig. 13). In the frog, staining was obtained with anti-86/82 only, and was localized both in intercalated discs (Fig. 14) and along and between the muscle fibres. Two antibodies gave staining of lizard heart: anti-86, which stained intercalated discs (Fig. 15); and anti-82/86, which, in addition, stained cross-striations, believed to be I-bands (Fig. 16). Two different staining patterns were obtained with three different antibodies in axolotl heart. Thus anti-86 and anti-205/230 gave punctate staining along the muscle fibres (Fig. 17), while 82/86 gave staining of cross-striations similar to that found in the lizard. Trout heart stained with two antibodies, anti-205/230 and anti-82/86. Each gave a similar somewhat irregular pattern as shown in Fig. 18.

**Skeletal and smooth muscle**

Staining was absent in the skeletal muscle of all species. Staining was also absent from chicken gizzard smooth muscle.

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Fig. 5. Section of rat liver stained with anti-205/230 showing punctate staining of the bile canaliculi.

Fig. 6. Section of axolotl liver stained with anti-205/230 showing staining of the bile canaliculi and small elements of the bile duct (b).

Fig. 7. Section of fish liver stained with anti-205/230 showing staining of the bile canaliculi.

Fig. 8. Section of lizard stomach stained with anti-86 showing punctate staining around the epithelial cells. Note the concentration of stain in the terminal bar regions of the gastric parietal cells shown in transverse section in the centre of the picture.

Fig. 9. Section of guinea pig stomach stained with anti-205/230 showing punctate staining between the gastric epithelial cells.

Fig. 10. Section of lizard intestine stained with anti-205/230 showing punctate staining between the intestinal epithelial cells, particularly concentrated in the terminal bar regions of the cells lining the lumen.
Fig. 17. Section of axolotl heart stained with anti-205/230 showing intense staining in punctate rows along the muscle fibres. No staining of intercalated discs was found. This pattern is also produced by anti-86. Anti-82/86 produced a similar pattern of staining to that of lizard heart (Fig. 18). No staining was found with antisera to the glycoproteins.

Fig. 18. Section of fish heart stained with anti-205/230 showing intense irregular staining believed to be intercalated discs. No staining of cross-striations was visible. This pattern was also found with anti-82/86. No staining was found with anti-86 or antisera to the glycoproteins.

Pillar cells of trout gill and pseudobranch

A brief description of these cells is necessary. They are structural elements that separate the epithelial cells of the secondary lamellae of the fish gill and pseudobranch, and form the boundaries of gill capillaries. Each consists of a roughly circular column having a flattened region or flange at each end, the latter being attached to the basement membranes of the epithelia on each side of the lamella. These basement membranes are joined by narrow cylinders of collagenous material, each of which is enclosed by the peripheral plasma membrane of a pillar cell. There are several such
columns arranged around the circumference of each pillar cell (see Hughes, 1977). This arrangement is shown in an electron micrograph depicting a transverse section of a single pillar cell (Fig. 19) where it can also be seen that fibrous mats are associated

Fig. 19. Transverse section through a pillar cell of trout pseudobranch showing fibrous mat (arrowheads) underlying the areas of cell membrane enveloping the collagen columns. Bar, 1 μm.

Fig. 20. Section of trout pseudobranch stained with anti-86/82 showing intense staining of the pillar cells. In longitudinal sections the staining appears as parallel lines while in transverse section (inset) it can be resolved into rings of staining distributed around the columns at the periphery of each cell. Bar, 20 μm.
**Distribution of desmosomal components**

with the cytoplasmic faces of the plasma membrane adjacent to the collagenous cylinders. Desmosomes join the flanges of adjacent pillar cells and are present between the epithelial cells of the gill. The structure of the pseudobranch is essentially similar to that of the gill except that the epithelial cells have become highly specialized and adjacent lamellae are joined together by interstitial material.

The pillar cells stain with anti-82/86 adjacent to the collagenous columns. This is evident both in longitudinal and transverse sections of the cells (Fig. 20). In transverse sections the staining is in circular profiles, which is consistent with staining around the columns. Anti-86 gave no staining of these cells, so the above pattern presumably reflects the distribution of an $82 \times 10^3 M_r$-like protein. These cells did not stain for prekeratin or any other desmosomal antigens. The epithelial cells of both gill and pseudobranch did, however, stain weakly with anti-205/230 and anti-prekeratin (see Billig et al. 1982 for details of anti-prekeratin antibody).

**DISCUSSION**

The picture of the desmosome that emerges from these studies is that of a highly conserved structure containing protein and glycoprotein components, which in turn show a considerable degree of conservation between tissues and species. Such evidence does not support the basic tenet of much research into the molecular mechanisms of cell adhesion; namely, that such mechanisms must be exclusive to tissue type and be species-specific (see, e.g., Obrink, Kuhlenschmidt & Roseman, 1977). According to the immunological criteria adopted here, the desmosomal plaque constituents are more extensively conserved than the glycoproteins. Moreover, there is some indication that the plaque constituents, or proteins related to them, may exist, primitively, in non-desmosomal locations where they are associated with simple adhesive junctions that do not possess the full structural complexity of desmosomes or hemidesmosomes. It also seems that the glycoproteins of the epidermis of different species may be more alike than those of epidermal and non-epidermal tissues of the same species.

**Table 4. Staining of heart from a range of species with antibodies to desmosomal components**

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All skeletal muscle negative.
All smooth muscle negative.
* These figures refer to the staining of I-bands.
These conclusions about the relative conservation of glycoproteins and plaque constituents are based on the relative intensity of fluorescent antibody staining. As shown in Tables 1–4 the difference in staining intensity between epidermal and non-epidermal tissue obtained with the anti-115 and anti-100 antibodies were absolutely consistent. Because of this consistency we feel that the $115 \times 10^3$ and $100 \times 10^3 M_r$ antigens from the epidermis of different species may have more epitopes in common than the same antigens in the epidermal and non-epidermal desmosomes of the same species. However, we must be cautious about this conclusion because diminished intensity of staining may represent either a decrease in number of qualitatively similar antigen molecules in non-epidermal desmosomes or a decrease in accessibility of these antigens to the antibody.

It should be stressed that an absence of staining with our anti-glycoprotein antibodies does not mean that the desmosomes of lower vertebrates are devoid of intercellular material. One of the most complete descriptions of desmosome structure is that of Lentz & Trinkaus (1971) for the desmosomes of the killifish, *Fundulus heteroclitus*. These desmosomes have a very prominent mid-line in the intercellular space, which we assume to be composed of glycoproteins that are not immunologically related to those of bovine nasal epithelium.

The specificity of our antisera as determined by immunofluorescent staining and immunoblotting has been discussed previously (Cowin & Garrod, 1983). However, here we have included a further antiserum that reacts with the $86 \times 10^3 M_r$ antigen only. This shows considerable differences in staining properties from anti-82/86. Thus, for example, the latter stained frog heart, trout heart and pillar cells whereas anti-86 did not. This seems to suggest that these tissues contain a protein that is immunologically related to the $82 \times 10^3$ but not to the $86 \times 10^3 M_r$ protein, and this in turn suggests that $82 \times 10^3$ and $86 \times 10^3 M_r$ proteins are not immunologically related. It is probable that we obtained antibodies against both proteins because there was some cross contamination of the $86 \times 10^3 M_r$ antigen eluted from polyacrylamide gels. Our conclusion that these two proteins are not immunologically related agrees with the interpretation of Cohen, Gorbsky & Steinberg (personal communication).

**Desmosomes and desmosomal antigens in heart**

Staining of heart tissue for desmosomal antigens has presented a variety of different patterns, clarification of which must await further electron microscopical and biochemical work. It should be pointed out that unlike the other tissues described here, heart is a mesenchymal structure and heart desmosomes are unusual in that they are not associated with cytokeratin, because heart does not stain with anti-prekeratin antibody (unpublished observations). Instead there is some suggestion they are associated with desmin, another type of intermediate filament (Franke et al. 1982; Kartenbeck, Franke, Moser & Stoffels, 1983).

Considering mammalian heart first, the bright staining of intercalated discs corresponds with the known distribution of desmosomes from electron microscopy (Chalice & Viragh, 1971). Similar staining of intercalated discs for the $230 \times 10^3$ and $205 \times 10^3 M_r$ proteins has been reported by Franke et al. (1982), who have demonstrated
that the antibody associates with the desmosomal plaques by immunoelectron microscopy. Here we show that mammalian heart desmosomes also stain for the other desmosomal antigens.

What is the significance of loss of staining for certain antigens in hearts of lower vertebrates? Although some comparative studies of the ultrastructure of the hearts of lower vertebrates have been published, precise details of the nature and distribution of intercellular junctions are not available. In some cases desmosomes have been reported to be located between muscle fibres (Somner & Johnson, 1979), which may account for the punctate staining found with the heart of chick, frog and axolotl. Our own preliminary studies of the ultrastructure of frog and axolotl heart suggest that true desmosomes are not present, but that there are primitive junctions between muscle fibres that do not show the same degree of development of intercellular materials. The staining of I-bands in lizard and axolotl heart must remain a matter for further investigation.

Desmosomal antigens in trout gills

The staining of pillar cells of fish gills must be attributed to the presence of a protein that is related to the $82 \times 10^3 M_r$ desmosomal protein, because staining occurs only with the antisera that react with the $86 \times 10^3$ and $82 \times 10^3 M_r$ proteins on immunoblots, and not with the antiserum that reacts with the $86 \times 10^3 M_r$ protein only. It is interesting to note that pillar cells, like heart, are of mesodermal origin (Morgan, 1974). The distribution of staining for the $82 \times 10^3 M_r$ antigen in parallel lines in vertical section, and circular profiles in transverse section, suggests that the antigen is distributed around the collagenous columns that pass through the pillar cells. The cytoplasmic fibrous mat surrounding these columns provides the most obvious candidate but this will have to be confirmed by immunoelectron microscopy, because the antigen could be situated in the plasma membrane instead. This fibrous mat does not have the structure of a hemidesmosome (Billig et al. 1982) and is certainly not a desmosome, because it is not located intercellularly. We suggest that in the fish gill the $82 \times 10^3 M_r$ antigen, or a related protein, has an existence outside and separate from the desmosome, contributing to a primitive type of substratum adhesive junction. At present we are attempting to isolate and characterize this protein from fish gill and thus determine to what degree it is related to the $82 \times 10^3 M_r$ desmosomal protein.

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