Early expression of desmosomal components during kidney tubule morphogenesis in human and murine embryos

D. R. GARROD and S. FLEMING*

Cancer Research Campaign, Medical Oncology Unit and Department of Pathology, University of Southampton, Southampton General Hospital, Southampton, SO9 4XY, UK

*Present address: Department of Pathology, University of Edinburgh, Teviot Place, Edinburgh, EH8 9AG, UK

Summary

Developing kidneys of human and murine fetuses have been stained with monoclonal antibodies to desmosomal proteins 1 and 2 (desmoplakins) (dp 1&2), desmosomal glycoprotein 1 (desmoglein) and a polyclonal antiserum to desmosomal glycoproteins 2 and 3 (desmocollins). All three antibodies stain the mesenchymal condensates that represent the first stage in kidney tubule development, indicating that desmosomal antigens are expressed very early in tubule morphogenesis. Desmosomal antigens are continuously expressed throughout the developing tubule being concentrated at the apical and basal regions of the lateral membranes of cells. Staining is also present in both visceral and parietal membranes of the developing Bowman’s capsule. In the mature tubule, desmosomal staining becomes restricted to a discontinuous apico-lateral ring around the cells. Staining is completely lost from the visceral membrane of the mature Bowman’s capsule (the podocytes) but persists in the parietal membrane.

At the condensate stage, staining for dp1&2 is much more intense than staining for simple epithelial keratin. Electron microscopy showed the presence of small (ca 0.1 μm) punctate junctions in the developing tubule. These may be immature desmosomes. No fully mature desmosomes such as are present in mature kidney were found.

The results suggest that desmosomal proteins and glycoproteins are involved in the early development of adhesive contacts between cells of the kidney tubule. The changing pattern of antigen expression, the loss of desmosomal staining from the podocytes and the immaturity of junctions suggest that desmosomal adhesion is labile during tubule morphogenesis, perhaps in order to facilitate changes of cell–cell contact.

Key words: Kidney development, epithelial morphogenesis desmosomes, cell-cell adhesion, intercellular junctions, cytokeratin, immunocytochemistry.

Introduction

Each kidney of the mouse contains about 2×10⁴ nephric tubules or nephrons, while in man the number is over 1×10⁶. Each nephron consists of a single layer of epithelial cells surrounding a central lumen and the epithelium is differentiated along its length into a number of clearly defined parts, Bowman’s capsule, proximal convoluted tubule, loop of Henle and distal convoluted tubule. Detailed descriptions of kidney tubule morphogenesis are given by Saxen and Wartiovaara (1966), Larson (1975) and Saxen (1987). A brief outline will be given here.

The tubules form from nephrogenic mesenchyme following induction by the ingrowing ureteric bud. First, an early condensation forms as loosely associated mesenchymal cells adhere together minimizing the intercellular space. This is followed by the formation of a pretubular condensation or vesicle stage in which the cells become radially elongated. Formation of a crevice dividing the potential glomerular epithelium from the future tubular epithelium causes the rudiment to become comma-shaped and initiates formation of the glomerulus by ingrowth of endothelial cells. This comma-shaped rudiment then becomes S-shaped as the tubular segment grows. As growth proceeds, a lumen is formed within the tubule as the epithelial cells polarise and the distal end of the tubule fuses with the ureteric bud to form the collecting duct. In cultured embryonic mouse kidney, development of the tubule rudiment to the S-shaped stage takes about 72 h.

The importance of changes in intercellular adhesiveness in formation of the early condensate and subsequent stages of tubule morphogenesis has been stressed (Saxen and Wartiovaara, 1966). A number of subsequent studies have investigated the appearance and distribution of adhesive extracellular matrix components and intercellular adhesion molecules in tubule morphogenesis. The early postinductive expression of basement membrane-specific components (laminin,
type IV collagen and basement membrane proteoglycan) in a punctate, unpolarized distribution changes to a basally polarized distribution as tubule morphogenesis proceeds (Ekblom et al. 1980, 1985; Ekblom, 1981) and is accompanied by the loss of mesenchymally associated fibronectin.

Expression of several cell adhesion molecules (CAMs) during kidney development has been studied. Both the neural and liver cell adhesion molecules (N-CAM and L-CAM) are expressed in early tubule development in the chick embryo (Thierry et al. 1982, 1984). In cultured embryonic mouse kidney, expression of uvomorulin, the mammalian homologue of L-CAM, begins 12 h after completion of induction, and is confined to the tubule and absent from glomeruli (Vestweber et al. 1985). N-CAM and uvomorulin are coexpressed for a short period during epithelial cell polarization, then N-CAM is lost (Klein et al. 1988). In the avian embryo, staining for A-CAM (adherens-junction-specific cell adhesion molecule) is present in the nephrogenic blastema and increases in amount at the time of condensation. It then disappears progressively along the tubule in a proximodistal gradient coincidently with the appearance of L-CAM (DuBand et al. 1988).

Desmosomes are adhesive intercellular junctions characteristically present in the majority of epithelia (Franke et al. 1981; Cowin and Garrod, 1983; Cowin et al. 1984). In mature epithelia, desmosomes appear to play a structural adhesive role, linking together the intermediate filament cytoskeletons of adjacent cells (Staehelin, 1974; Garrod, 1985; Cowin et al. 1985). Desmosomes are present in the early mammalian embryo between the trophodermal cells of the blastocyst (Ducibella et al. 1975) and in the area opaca of the early chick embryo (Overton, 1962; Garrod et al. 1989). However, their role in embryonic development and epithelial morphogenesis has not been defined. The formation and stability of desmosomes and desmosomal components in cultured kidney epithelial cell lines has been studied by a number of groups (Mattey and Garrod, 1986a,b; Penn et al. 1987a,b; Pasdar and Nelson, 1988a,b). In this paper, we describe the expression and distribution of desmosomal components in developing human and murine kidney tubules by staining with well-characterized monoclonal and polyclonal anti-desmosomal antibodies. We also compare the appearance of staining for desmosomes and that of low relative molecular mass keratins (50000, 43000 and 39000), and present some electron microscopical information on intercellular junctions in developing kidney epithelium. Our results suggest an early and continuing contribution of desmosomal adhesion to kidney tubule morphogenesis.

Materials and methods

Kidneys

Human fetal kidneys of various gestational ages ranging from 12 to 32 weeks were obtained at autopsy. In the human embryo, kidney tubule development begins at 5 weeks of gestation and continues until 32–36 weeks (Osathanondh and Potter, 1963), so that kidneys at all intervening stages exhibit all stages of nephron development. Kidneys similarly showing all stages of nephron development were dissected from mouse embryos of various stages between 13 and 17 days of gestation.

For immunocytochemistry, kidneys were snap frozen in liquid nitrogen and sectioned at 4 μm on a cryostat and some human kidneys were fixed in 10% neutral buffered formalin, embedded in paraffin wax and sectioned at 4 μm on a conventional microtome. Before staining, frozen sections were allowed to dry on slides in air at room temperature for at least two hours and then were used immediately or stored desiccated at −20°C until required.

Antibodies

Three antibodies to different desmosomal components were used in this study, two monoclonal and one polyclonal. For a description of the nomenclature of desmosomal components and their localization within mature desmosomes, see Miller et al. (1987) and Garrod et al. (1989). Monoclonal antibody 11-5F reacts with desmosomal proteins 1 and 2 (dp 1&2) (desmoplakins) in frozen sections, and has been described and characterized by Parrish et al. (1987). Monoclonal antibody 32-2B reacts with desmosomal glycoprotein 1 (dg1) (demi-glen) in formaldehyde-fixed, paraffin-embedded sections, and has been described and characterized by Vilela et al. (1987). Subsequent studies indicate that 32-2B reacts with the cytoplasmic domain (trypsin-protected in living cells) of dg1, which is a transmembrane glycoprotein (M. J. Vilela – unpublished observations). Guinea-pig polyclonal antiserum to desmosomal glycoproteins 2 and 3 (dg2&3) (desmocollins) has been described and characterized (Cowin and Garrod, 1983).

Staining of keratin in both fixed and frozen sections was with monoclonal antibody CAM 5.2, which labels low relative molecular mass keratins (50000, 43000 and 39000) of simple epithelial cells (Makin et al. 1984).

Immunocytochemistry

Monoclonal antibodies were used as neat supernatants or diluted 1:5, while polyclonal guinea-pig antiserum was used at dilutions of 1:50 or 1:100. Both frozen and fixed sections were treated with 0.1 % trypsin for 30 min at 37°C before staining. Sections were counterstained with haematoxylin. Double staining of sections with monoclonal antibodies 11-5F and CAM 5.2, using an alkaline phosphatase conjugate to develop the former and a peroxidase conjugate to develop the latter, was carried out on frozen sections as described by Mason et al. (1981). No counterstain was used.

Double fluorescent staining with monoclonal antibodies was carried out as follows. A series of sections was stained with double dilutions of one of the primary antibodies (11-5F or CAM 5.2) and a standard working concentration (recommended by the manufacturers) of a fluorochrome-conjugated (fluorescein or rhodamine) second antibody, in order to determine the minimum concentration of first antibody that would give adequate detectable staining. Once this concentration had been determined, another series of sections was stained using this concentration of the monoclonal but using doubling dilutions of the fluorochrome conjugate. These sections were then double stained using a
Figs 1-4. Staining with monoclonal antibody, 11-5F, to desmosomal proteins 1&2 (desmoplakins) by the avidin-biotin peroxidase technique of stages in the development of the human kidney tubule from a fetus of 22 weeks gestation. Fig. 1. Early condensate (arrow) and ureteric bud (ub). Staining is absent from the uncondensed mesenchyme (m). Fig. 2. S-shaped stage showing staining in the developing parietal (arrowhead) and visceral (arrow) membranes of Bowman’s capsule and in the developing tubule (t). Fig. 3. Immature Bowman’s capsule and tubule (t). Note the presence of staining especially in both the parietal (arrowhead) and visceral (arrow) membranes of the capsule. Fig. 4. Mature glomerulus, Bowman’s capsule and proximal tubule (pt). Note the presence of staining in the parietal membranes (arrowhead) but absence of staining from the region of the glomerulus which includes the mature podocytes of the visceral membrane (arrow). Note also the strongly localised distribution of staining in the proximal tubule. Bars, 25 μm.

Fig. 5. Double staining of a ureteric bud (ub) and an early vesicle (v) stage of tubule development from a human fetal kidney at 22 weeks gestation with monoclonal antibodies CAM 5.2 and 11-5F to simple epithelial keratin and dp1&2 respectively. CAM 5.2 is localised with peroxidase (brown) and 11-5F with alkaline phosphatase (blue). The section is not counter stained. The CAM 5.2 staining in the ureteric bud is very intense and masks staining for the desmosomal antigen, but in the vesicle staining for the desmosomal antigen greatly exceeds that for keratin. Bar, 25 μm.
working concentration of a second anti-mouse conjugate bearing the other conjugate. In this way, conditions were determined so that the first monoclonal was detected by the first fluorescent conjugate but not by the second. Double staining was then carried out under these conditions, but applying the second monoclonal antibody between the two fluorochrome conjugates. An example of this technique and its controls are shown in Figs 6, 7, 8 and 9.

Fluorescent anti-mouse antibodies were obtained from Amersham and avidin-biotin reagents from Vector Laboratories or Dakopatts.

**Electron microscopy**

Whole fetal mouse kidneys were fixed in 2.5% glutaraldehyde in cacodylate buffer at pH 7.4. After fixation they were washed in 100 mM-cacodylate buffer, pH 7.4, containing 230 mM-sucrose and 2 mM-CaCl₂. Washing was followed by postfixation in 2% OsO₄ for 2h and staining with 1.5% uranyl acetate. Specimens were embedded in Spurr resin and sections examined on a Phillips 201 electron microscope.

**Results**

**Desmosomal components in kidney tubule morphogenesis**

Sections of human and murine fetal kidneys of various gestational ages were stained by the avidin–biotin peroxidase technique with monoclonal antibodies to dp1&2 and dgl, and a polyclonal antiserum to dg2&3, counterstained with haematoxylin and examined by light microscopy. Within the limits of this technique, no differences between the timing of expression and distribution of the different desmosomal components were detectable, nor were there differences between kidneys of different age or species with regard to the details of antigen expression in relation to tubule morphogenesis. The detailed situation will therefore be described in relation to staining with anti-dp1&2 in human fetal kidney. Slides showing staining for the other antigens in human and murine kidneys are available for inspection.

No staining of any mesenchymal elements was found with desmosomal antibodies. Ureteric buds showed strong staining concentrated towards the apicolateral surfaces of the cells with some staining also on the more basal regions of lateral membranes (Fig. 1, Fig. 9).

The boundaries of cells in the early mesenchymal condensates showed significant staining which was fairly evenly distributed on these unpolarized cells (Fig. 1). Thus expression of desmosomal antigens occurs at the earliest stage of kidney morphogenesis.

At the comma- and S-shaped stages of development, desmosomal antigen staining was present in all epithelial parts of the developing tubule and renal capsule, including both parietal and visceral epithelia of the latter (Fig. 2). Staining was somewhat more intense in the apical and basal regions of the cells but was also present along most of the mutually adherent cell boundaries. (A similar distribution of antigen was also found at the vesicle stage, which immediately follows the early condensate, as shown by fluorescent staining in Fig. 9.)

At a slightly later stage, when Bowman’s capsule and the glomerulus are more developed but still immature, staining was still present in all parts of the epithelium, including the visceral epithelium of the glomerulus, the future podocytes (Fig. 3).

The distribution of desmosomal staining in the mature Bowman’s capsule and tubule was much more restricted than during morphogenesis. In the renal corpuscle, staining was present only between the lateral membranes of the parietal epithelial cells of Bowman’s capsule and absent from the visceral, podocyte epithelium (Fig. 4). In the tubule, the antigens were largely confined to a narrow apicolateral zone, which appeared as a discontinuous ring around the cell in tangential section (Fig. 4).

**Comparison of expression of dp1&2 and keratin in epithelial condensates**

In order to compare the expression of desmosomal antigens with that of another epithelial marker that has been well studied in this system, double staining of developing mouse kidneys for low relative molecular mass keratins with monoclonal antibody CAM 5.2 and dp1&2 was carried out.

In Fig. 5 such double staining using the combined immunoperoxidase–alkaline phosphatase technique is shown. The figure shows absence of any staining in the mesenchyme, intense staining for keratin in the ureteric bud and intense staining for dp1&2 in the early vesicle. This result may have indicated that expression of desmosomal antigens precedes expression of keratin in early tubule morphogenesis. However, it is clear that intense staining for one antigen may mask weaker staining for another. In order to resolve this issue, we used double immunofluorescent staining with the two monoclonal antibodies, thus enabling the different antigens to be viewed independently with different fluorochromes.

In order to validate the technique, staining conditions were first carefully established as described in Materials and methods. Figs 6 and 7 show a control experiment in which desmosomal staining with 11-5F was detected using a rhodamine-conjugated second antibody, and no desmosomal staining was revealed by a fluorescein-conjugated second antibody applied after the first conjugate. Figs 8 and 9 shows the result of double staining with 11-5F and CAM 5.2 under these carefully controlled conditions. The ureteric bud in the centre of the figure shows filamentous staining for keratin, while staining for dp1&2 is intense in the apicolateral regions of the cells but punctate elsewhere. In the early vesicle to the left of the ureteric bud, strong punctate staining for dp1&2 is present but the staining for keratin is less extensive and also punctate rather than fibrillar. Some co-localization of staining by the two antibodies is evident. Such staining was typical of the early condensate and vesicle stages. No cases were found in which either antigen was expressed in complete absence of the other.

**Electron microscopy**

To our knowledge, desmosomes have not been de-
Figs 6 and 7. Control experiment for double immunofluorescent staining with monoclonal antibodies CAM 5.2 and 11-5F. The section was reacted with 11-5F, followed sequentially by rhodamine-conjugated anti-mouse IgG and fluorescein-conjugated anti-mouse Ig. Viewed with fluorescein filters no staining is seen (Fig. 6) but with rhodamine filters the punctate desmosomal staining of the kidney tubule is evident in the same field of view (Fig. 7). Bar, 20 μm.

scribed in developing kidney tubules. Since desmosomal components are clearly expressed from the very earliest stages of tubule formation, we have looked for intercellular junctions in the mouse embryonic kidney by electron microscopy. Two distinct types of junctions were found between developing tubule cells. The first were characterized by dense plaques closely associated with the cytoplasmic faces of plasma membranes (Fig. 10). These were most extensive in the apicolateral regions of cells of the developing tubule where, in some sections, they extended for as much as 2 μm and showed branching at regions where several cell processes made contact (Fig. 10). These junctions were present from the vesicle stage onwards but were less extensive in the very early stages.

The second type of junction was punctate consisting of small but clearly defined, paired, dense plaques occupying 0.1 μm or slightly less of plasma membrane length (Figs 11 and 12). In some cases, there was indication of association of cytoskeletal elements with these plaques. In most cases, the intercellular space between the plaques showed the distinct presence of dense material compared with non-junctional regions (Figs 11 and 12).

These punctate junctions were present in all parts of the developing tubule including the visceral epithelium of the glomerulus. They were found along the lateral membranes between tubule cells at the S-shaped stage with no obvious concentration in the apical region. No junctions with the complete structure of fully mature desmosomes were found.

Discussion

The initiation of epithelial differentiation leading to kidney tubule formation involves the early expression of a number of characteristically epithelial antigens, including laminin, type IV collagen, basement membrane proteoglycan, keratin and uvomorulin (Ekblom et al. 1980, 1985; Ekblom, 1981). To these may now be added components immunologically related to the desmosomal proteins and glycoproteins, dp1&2, dg1 and dg2&3. Desmosomal components are expressed at the early condensate stage and subsequently throughout the developing tubule.

Expression of desmosomal components so early in epithelial morphogenesis is surprising because desmosomes are usually regarded as stabilizing elements in epithelial organisation, more prevalent in tissues, such as epidermis, where strong intercellular adhesion is required to resist abrasion (Staehelin, 1974). This idea has been formalised as the precedence hypothesis which suggests that morphogenesis is dependent upon the modulation of CAMs and that junctions appear later (Edelman, 1988). From this point of view, it might be expected that desmosomal expression would be a late event in epithelial morphogenesis, bringing stability to cell contact. In kidney tubule development, on the contrary, it appears that desmosomal components may play a role in very early morphogenesis, probably contributing to the establishment of tighter cell adhesion between cells in the early condensate. Adhesion in mature epithelia is complex, involving contributions from a number of different molecular mechanisms (Garrod, 1985; 1986a, b). The expression of desmosomal components together with a number of CAMs (see Introduction) in the kidney tubule rudiment shows that complexity is already present in early morphogenesis. It will be of great interest to determine what adhesion mechanisms are expressed in other early epithelial structures.

Following initial condensation, plasticity of adhesion between the proliferating cells of the tubule rudiment is required in order that epithelial morphogenesis may proceed. The changing pattern of desmosomal staining during morphogenesis suggests that the expression of desmosomal components may be labile and thus contribute to this adhesive plasticity. We show that in the mature kidney tubule, desmosomal expression appears restricted to the apicolateral cell borders, but early staining is much more widely distributed on the lateral cell borders, extending as far as the basolateral cell interfaces. This changing pattern of expression of desmosomal antigens resembles that previously described for dp1&2 staining in developing mammary gland epithelium by Dulbecco et al. (1984).

In one part of the tubule, the visceral membrane of
Figs 8 and 9. Double immunofluorescent staining human kidney from fetus of 16 weeks gestation with monoclonal antibodies CAM 5.2 and 11-5F. CAM 5.2 is visualized with a fluorescein conjugate (Fig. 8) and 11-5F with a rhodamine conjugate (Fig. 9). The ureteric bud (ub) shows strong filamentous staining for keratin (Fig. 8) and polarized staining for dp1&2. The early vesicles (v), however, show much less intense staining for keratin. The vesicle on the left in particular shows very weak keratin staining which in some areas (e.g. arrows) shows similar distribution to the stronger desmosomal staining. Bar, 20 μm.
Bowman's capsule, transient expression directly indicates turnover of desmosomal components during development. We have shown previously (Fleming and Symes, 1987; Fleming and Jones, 1987) that the visceral epithelium also shows transient expression of keratin. Thus the podocytes of the mature Bowman's capsule pass through a transient epithelial phase during their development, and then lose epithelial characteristics as they become more highly specialized. Perhaps turnover of desmosomal components also takes place in other parts of the developing tubule epithelium, but expression continues throughout development, becoming stabilized and spatially localized on tubule maturation. Loss of epithelial characteristics, including keratin and desmosomal staining, is a feature of some sarcomatoid renal carcinomas, a feature compatible with cellular dedifferentiation (Holthofer et al., 1983; Fleming et al., 1989). We note that expression of dp1&2 in the outer (parietal) membrane of mature Bowman's capsule found here is contrary to the results of Bachman et al. (1983) on rat kidney.

Desmosomes are punctate junctions. Electron microscopy of developing kidney tubules revealed punctate junctions with cytoplasmic plaques and dense intercellular material. However, these did not possess the complete structure of mature desmosomes, lacking a discernible midline and showing scantly association of cytoskeletal elements. The distribution of these junctions was consistent with that of desmosomal staining, and they were present in early mesenchymal condensates and immature visceral epithelium as well as in other parts of the tubule. A possible interpretation of these junctions is that they represent immature desmosomes, resembling structures found by others during desmosome formation (Lentz and Trinkaus, 1973; Dembitzer et al., 1980; Mattey and Garrod, 1986). Lack of a fully mature structure may reflect the lability of these junctions. In the adult kidney tubule, fully mature desmosomes are present. We have not yet determined precisely when this junctional maturation takes place. Freeze-fracture studies of kidney tubule development have shown that tight junctions (zonulae occludentes) appear in immature form at the early condensate stage, and increase in complexity as the tubule develops (Minuth et al., 1981).

An alternative view is that these punctate junctions may represent so-called 'type II desmosomes' as described in intestinal epithelium by Drenckhahn and Franz (1986). 'Type II desmosomes' are associated with actin filaments rather than intermediate filaments, and contain α-actinin rather than the major desmosomal proteins and glycoproteins. An immunoelectron microscopic study has been initiated in order to distinguish between these possible interpretations.

The more extensive plaque-bearing junctions found in the developing tubule resemble junctions of the zonula adherens type similar to those described in various developing tissues in the chicken by Duband et al. (1988).

The initial condensation of mesenchyme is accompanied by changes in the expression of intermediate filament cytoskeletal components, vimentin being replaced by low relative molecular mass keratins in renal vesicles (Holthofer et al., 1984; Lehtonen et al., 1985; Fleming and Symes, 1987; Fleming and Jones, 1987). As noted above, expression of keratins is transient in the visceral glomerular epithelium (Fleming and Symes, 1987; Fleming and Jones, 1987), only vimentin being
found in the mature epithelium (Holthofer et al. 1984; Bachmann et al. 1983). In the remainder of the tubular epithelium, expression of keratin persists.

The distribution of keratin in the early condensate was found to be punctate or focal rather than filamentous, as noted previously (Fleming and Symes, 1987). Here we have found keratin and desmosomal staining were colocalized in some punctae in the early condensate. This appears to indicate that association of keratin with desmosomal plaque components may precede keratin filament formation in this tissue. This resembles the situation found during calcium-induced desmosome formation in a cultured rat mammary epithelial cell line (Bologna et al. 1986), where desmosomes are believed to act as organizing centres for keratin filament assembly. More recent work on tissue culture cells suggests, however, that keratin filament assembly may originate at the nuclear membrane (Albers and Fuchs, 1989).

Study of a single example of nephroblastoma (Wilm's tumour) by fluorescent staining with anti-keratin and anti-desmoplakin antibodies showed that desmosomal plaques were present in the absence of keratin in the tumour tissue (Denk et al. 1985). The authors suggested that desmoplakins are an early differentiation marker in this tissue (kidney epithelium), and their results may suggest that desmoplakin expression might be expected to precede keratin expression in normal development. Although we have not found situations in the developing tubule where expression of desmosomal components occurs in the absence of keratin, expression of the former certainly appeared to exceed the latter in some early condensates. We therefore leave open the question of whether desmosomal expression slightly precedes keratin expression in normal development. In vitro experiments where the onset of induction can be timed more precisely may provide a definitive answer.

In conclusion, induction of kidney mesenchyme by the ureteric bud appears to provide a general signal for epithelial differentiation resulting in the expression of several characteristically epithelial components including desmosomal proteins and glycoproteins. Expression of desmosomal components and CAMs results in increased intercellular adhesiveness and consequent condensation. Lability of expression of desmosomal components is consistent with the requirement for
plasticity of adhesion during subsequent epithelial morphogenesis. Localization and stabilization of the pattern of desmosomal expression accompanies maturation of the tubule structure.

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References


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