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Monoclonal antibodies to sperm surface antigens of the brown alga *Fucus serratus* exhibit region-, gamete-, species- and genus-preferential binding

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Abstract. A panel of twelve monoclonal antibodies (MAbs), designated FS1 to FS12, have been raised against surface antigens of *Fucus serratus* sperm. The antibodies were selected on the basis that they show region-, gamete-, species- or genus-preferential binding. Indirect immunofluorescence shows that the antigens bound by the MAbs are distributed non-randomly over the cell surface. Seven MAbs (FS1, FS3, FS4, FS6, FS8, FS9, FS10) bind antigens located primarily on the cell body, while the others (FS2, FS5, FS7, FS11, FS12) bind antigens located primarily on the anterior flagellum. Of the MAbs that label the anterior flagellum, FS2, FS5, FS7 and FS12 form a 'halo' at the perimeter of the flagellum. Electron microscopic-immunogold studies indicate that the 'halo' results from labelling of the mastigonemes, as opposed to the flagellar plasmamembrane. Gamete-preferential binding of antibodies was detected using an enzyme-linked immunosorbent assay with egg membrane vesicles. Eight of the MAbs bind sperm antigens not common to eggs, though FS2, FS4, FS5 and FS9 bind antigens present on both sperm and eggs. In studies of species- and genus-specificity FS2, FS3, FS5, FS6, FS7, FS8, FS10, FS11 and FS12 exhibit genus-preferential binding, labelling sperm of *F. serratus* and *F. vesiculosus* more intensely than that of *Ascophyllum nodosum*. Only FS10 showed marked species-preferential binding, labelling sperm of *F. serratus* much more intensely than that of *F. vesiculosus*.

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Abbreviations: Au-GAMIG = gold-conjugated goat anti-mouse immunoglobulin; ELISA = enzyme-linked immunosorbent assay; EM = electron microscope; FITC-RAMIG = fluorescein-isothiocyanate-conjugated rabbit anti-mouse immunoglobulin; IIF = indirect immunofluorescence; MAb = monoclonal antibody

Key words. Cell surface – Fertilisation (recognition) – *Fucus* – Mastigoneme – Monoclonal antibody – Sperm (surface)

Introduction

Sexual reproduction in marine brown algae such as *Fucus serratus* is achieved by the liberation of naked gametes into the sea. The fertilisation process, which is species-specific, involves the interaction of large, non-motile eggs with small, biflagellate, motile sperm. The eggs secrete a pheromone to attract the sperm, after which recognition of the gametes occurs followed by fusion of sperm and egg membranes (Callow 1985). The species specificity of sperm-egg recognition is not mediated by the pheromone (Muller and Seferiadis 1977), but evidence from experiments using lectins, glycosidases, gamete membrane fractions and polyclonal antiserum raised against the sperm, strongly indicates that it resides at the plasmamembrane and is probably mediated by surface saccharides (Bolwell et al. 1979, 1980; Catt et al. 1983; Vithanage et al. 1983). Unfertilised *Fucus* eggs are completely apolar and differ from eggs of animals (e.g. sea urchin, mammals) in that they are bounded by a plasmamembrane only, without having a jelly coat. The biflagellate *Fucus* sperm, however, is a highly differentiated structure. The shorter, anterior flagellum bears mastigonemes arranged bilaterally (Manton and Clarke 1951) whereas the longer, posterior flagellum lacks such hairs. The anterior end of the sperm body is extended into a flattened structure known as the proboscis, which has an interior framework of thirteen concentric microtubules which aggregate in the region of the root

of the anterior flagellum (Manton and Clarke 1951).

One characteristic of sperm cells of many animals is that specific surface antigens are localised to particular regions (domains) of the cell (e.g. head, tail) and the molecules in these separate domains often have specific functions related to the fertilisation process (Primakoff and Myles 1983; Saxena et al. 1986). Such regional heterogeneity has been revealed most convincingly by the use of monoclonal antibodies (MAbs), raised against cell surface components, which have been used to localise antigens through techniques such as indirect immunofluorescence (IIF) and electron microscope-immunogold labelling. The primary aim of the present work was to investigate the spatial organisation of surface antigens of *Fucus* sperm, and particularly to determine whether such antigens occur in discrete regions or are expressed over the entire cell. Monoclonal antibodies were prepared against cell surface antigens of *F. serratus* sperm, and results of localisation studies showed that some antigens are highly concentrated in particular regions whereas other antigens occur over the entire cell surface. The MAbs were also tested for cross-reactivity with *F. serratus* eggs and with sperm of *F. vesiculosus* and *Ascophyllum nodosum*. In these tests some of the MAbs showed gamete-, species- or genus-preferential binding.

Materials and methods

Plant material. Sexually mature plants of *F. serratus, F. vesiculosus* and *Ascophyllum nodosum* were collected from Watchet, Somerset, UK (Crothers 1976) (or occasionally from Anglesey, UK), sexed by microscopic examination of hand sections of the receptacles and stored separately at 4° C in ventilated plastic trays for up to 17 d. Gametes were released from excised receptacles as described by Callow et al. (1978). Egg membrane vesicles were prepared as described by Bolwell et al. (1980) and the protein content, of the crude fraction (30000-g pellet) used for enzyme-linked immunosorbent assay (ELISA), was determined by the Bio-Rad Bradford test according to the suppliers instructions (Bio-Rad, Watford, Herts., UK).

Immunisation and production of MAbs. Six- to eight-week old Balb-c mice were immunised intraperitoneally with 10^8 live sperm cells of *F. serratus* in 200 µl of sea water, similarly boosted three weeks later, and given a final intravenous injection (10^8 cells in 200 µl sea water) one to six months later, 4 d before the fusion. Fusions between immune spleen cells and NSO-myeloma cells, and the cloning of the appropriate hybridomas, by limiting dilution, were performed as described by Galfre and Milstein (1981).

Screening procedures. Culture supernatants were screened by ELISA and IIF. The ELISA was performed on Immulon I microtitre plates (Gibco, Uxbridge, UK) coated in sequence with each of the following: (i) 100 µl per well of poly-L-lysine (10 µg·ml^{-1}) for 2 h; (ii) 100 µl per well of a sperm suspension ($2 \cdot 10^6$–$5 \cdot 10^6$ cells·ml^{-1} sea water containing 10 mM azide), or 50 µl per well of crude egg membrane vesicle (at 50 µg protein·ml^{-1}), to which was added, after 2 h at 4° C, an equal volume of 0.5% (w/v) glutaraldehyde in sea water for three min; (iii) 250 µl per well of blocking solution containing 10 mg·ml^{-1} bovine serum albumin (BSA), 100 mM glycine and 0.02% (w/v) azide in phosphate-buffered saline (PBS), for more than 12 h at 4° C. Between steps, plates were washed twice with 250 µl 0.05% (v/v) Tween 20 (Sigma, Poole, Dorset, UK) in PBS, and stored with blocking solution at 4° C. For screening the hybridoma supernatants, wells received each of the following in sequence: (i) 50 µl of culture supernatant for 2 h at 20° C; (ii) 4 × 250 µl 0.05% (v/v) Tween 20 in PBS, with blotting between washes; (iii) 100 µl of alkaline-phosphatase-conjugated rabbit anti-mouse immunoglobulin (RAMIG; Dakopatts, High Wycombe, Bucks., UK) diluted 1/600 with 0.5 mg·ml^{-1} BSA in PBS, for 2 h at 20° C; (iv) 4 × 250-µl washes as above; (v) 100 µl of 1 mg·ml^{-1} *p*-nitrophenylphosphate freshly dissolved in 9.7% (v/v) diethanolamine buffer pH 9.8 for 1 h at 20° C; (vi) 50 µl of 3 M NaOH was added to stop the reaction. Optical density was read at 405 nm.

Indirect immunofluorescence was performed on multi-well microscope slides (Flow Laboratories, Rickmansworth, Herts., UK). To a suspension of sperm (10^7·ml^{-1}) an equal volume of fixative (8%, w/v, formaldehyde; 0.4%, w/v, glutaraldehyde in 100 mM 1,4-piperazinediethanesulfonic acid [Pipes] pH 7.1) was added and the mixture incubated at 4° C for 15 min. Cells were then pelleted in a microfuge (11600·*g* for 10 s), gently resuspended in PBS using a Gilson pipette, washed a further two times with PBS, finally resuspended in PBS at 10^6 cells·ml^{-1}, and then dispensed on to the slides at 10 µl per well. Slides were allowed to dry at 20° C for 1 h and then stored on silica gel at 4° C, although in some experiments fixed cells were allowed to adhere to slides coated with poly-L-lysine (10 µg·ml^{-1}) and were not subsequently dried down. To screen MAbs, slides were washed twice in PBS and 10 µl of culture supernatant added to each well. Controls received 10 µl of a hybridoma supernatant which contained a mouse monoclonal antibody raised against rat bone cells (data not shown). After 45 min incubation in a humid chamber at 20° C, slides were washed three times in PBS and then 10 µl of FITC-RAMIG (fluorescein-isothiocyanate-conjugated rabbit anti-mouse immunoglobulin; Dakopatts) was added to each well. After a further 45-min incubation, as above, slides were rinsed as above, then briefly in distilled water, and each well was mounted with 5 µl of mounting medium (glycerol/PBS 9:1 adjusted to pH 8.6). Sperm were observed with a Zeiss (Oberkochen, FRG) Axioplan Epi-fluorescence microscope fitted with Zeiss FITC excitation filters and photographs were taken with a Contax 139 Quartz camera, using Ilford HP4 (400 ASA) film.

Immunogold labelling. To a suspension of sperm cells (10^8·ml^{-1}) in sea water an equal volume of fixative (4%, w/v, glutaraldehyde in 100 mM Pipes pH 7.1) was added and the mixture incubated for 15 min at 4° C. Cells were pelleted and washed as above and finally resuspended in PBS at 10^7 cells·ml^{-1}. One hundred microlitres of tissue culture supernatant was added to 50 µl of sperm suspension. After 45 min incubation at 20° C, the cells were pelleted in a microfuge (11600·*g* for 1 min), resuspended in 1 ml PBS, similarly washed again and then resuspended in 50 µl of gold-conjugated goat anti-mouse immunoglobulin (15 nm, Au-GAMIG; Janssen Life Science Products, Wantage, Oxon., UK) which had been diluted 1/20 in PBS containing 0.5% BSA. After 45 min incubation the cells were washed twice, as above, and finally resuspended in 25 µl PBS. Controls received a MAb which had been raised against rat bone cells.

Negative staining and electron microscopy. A droplet (approx 3 µl) of the suspension of gold-labelled sperm cells was placed on a formvar-coated copper grid (200 mesh) for 5 min and the grid gently dried with velin tissue. Cells were negatively stained for 20–30 s with a droplet of ammonium molybdate, which was gently removed with velin tissue, and the grids allowed to dry. Sperm cells were observed with a Philips (Eindhoven, The Netherlands) 301 Electron microscope.

Immunoglobulin-class determination. Immunoglobulin class and subclass was determined by Ouchterlony immunodiffusion using a typing kit (Serotec, Bicester, Oxon., UK) according to the suppliers instructions.

Results

Screening and selection of hybridomas. Hybridoma culture supernatants were initially tested for anti-sperm (*F. serratus*) antibody by ELISA, and by IIF with *F. serratus* sperm (to screen for region-specific binding). Positive supernatants were then screened by IIF and ELISA with sperm of *F. vesiculosus* and *A. nodosum* (to screen for species- and genus-specific binding) and by ELISA with *F. serratus* egg membrane vesicles (to test for gamete-specific binding). From three fusions, approx. 1000 hybridoma cultures were screened, of which approx. 400 produced antibody to *F. serratus* sperm. Twelve cell lines were selected which produced antibody to *F. serratus* sperm and which showed region-, gamete-, species- or genus-preferential binding and these cell lines were cloned by limiting dilution. The binding characteristics and immunoglobulin subclasses of these MAbs, designated FS 1–12, are listed in Table 1. The MAbs were judged to be against cell surface antigens since they labelled well-fixed sperm which had not been dried down either on to slides for IIF or on to microtitre plates for ELISA.

Distribution of sperm surface antigens on the basis of immunofluorescence. Under the light microscope and electron microscope (EM) each sperm appears as a droplet-shaped (6 µm) biflagellate cell, with the mastigoneme-bearing anterior flagellum shorter than the posterior flagellum (Figs. 1, 2a). Indirect immunofluorescence was performed routinely on sperm cells which were fixed and dried down on to slides, but the twelve MAbs chosen for study showed the same binding patterns on both dried and non-dried preparations. The MAbs can be classified into two broad groups (Fig. 1, Table 1) – those which bind antigens that are concentrated on the sperm cell body (FS1, FS3, FS4, FS6, FS8, FS9, FS10) and those which bind antigens which are more concentrated on the anterior flagellum (FS2, FS5, FS7, FS11, FS12). Within these two groups there are further variations in the MAb-binding patterns. In the first group, FS1 binds almost exclusively to the sperm body (Fig. 1b, Table 1), FS9 and FS10 label the body more intensely than either of the flagella though the labelling of the flagella is greater than background (Fig. 1j, k, Table 1), and FS3, FS4, FS6, and FS8 bind preferentially to the sperm body though they also label the flagella (Fig. 1d, e, g, i, Table 1). In the second group the MAbs (FS2, FS5, FS7, FS11, FS12) all primarily label the anterior flagellum, but differ in the extent to which they bind the rest of the sperm cell (Fig. 1, Table 1). Thus, whereas FS11 binds exclusively to the anterior flagellum (Fig. 1l), FS2 and FS5 label the body very slightly, though not the posterior flagellum (Fig. 1c, f), and FS7 and FS12 label the whole cell (Fig. 1h, m).

Of those antibodies, within both groups, that label the anterior flagellum, two distinct immunofluorescent binding patterns are observed (compare Fig. 1n, o with p). FS2, FS5, FS7 and FS12

Table 1. Binding patterns of MAbs FS1–FS12 to gametes of *Fucus serratus*

MAb	Immunofluorescence of sperm[a]			ELISA[b]	
	Anterior flagellum	Body	Posterior flagellum	Sperm	Egg
FS4 (IgM)	++	+++	++	+++	+
FS3 (IgM)	+	++	+	++	−
FS6 (IgG1)	+	++	+	++	−
FS8 (IgM)	+	++	+	++	−
FS9 (IgG2b)	+	+++	+	+	+
FS10 (IgM)	+	+++	+	++	−
FS1 (IgM)	−	++	−	+−	−
FS7 (IgG1)	+++ (H)	++	+	++	−
FS12 (IgG1)	++ (H)	+	+	++	−
FS5 (IgM)	+++ (H)	+−	−	++	+−
FS2 (IgG1)	++ (H)	+−	−	++	+
FS11 (IgM)	++	−	−	+++	−
Anti-egg MAb (11.3A9.C2)	+	+	+	+	+++
Control MAb	−	−	−	−	−

[a] Fixed sperm cells mounted on glass slides were incubated with MAb and then FITC-RAMIG. The intensity of fluroescence is recorded as follows: − absent; +− very weak; + weak; ++ intermediate; +++ strong

[b] Whole sperm cells, or egg membrane vesicles, fixed on to ELISA plates, were incubated with MAb, then alkaline-phosphatase-labelled RAMIG and then a solution of *p*-nitrophenyl-phosphate. The intensity of the colour that developed for each MAb has been scored as follows: − no colour; +− slight colour (0.1–0.2 O.D. units); + intermediate (0.2–0.5); ++ strong colour (0.5–1.2); +++ very strong colour (>1.2). The anti-egg MAb was included to validate the egg vesicle ELISA. The control MAb was raised against rat bone cells

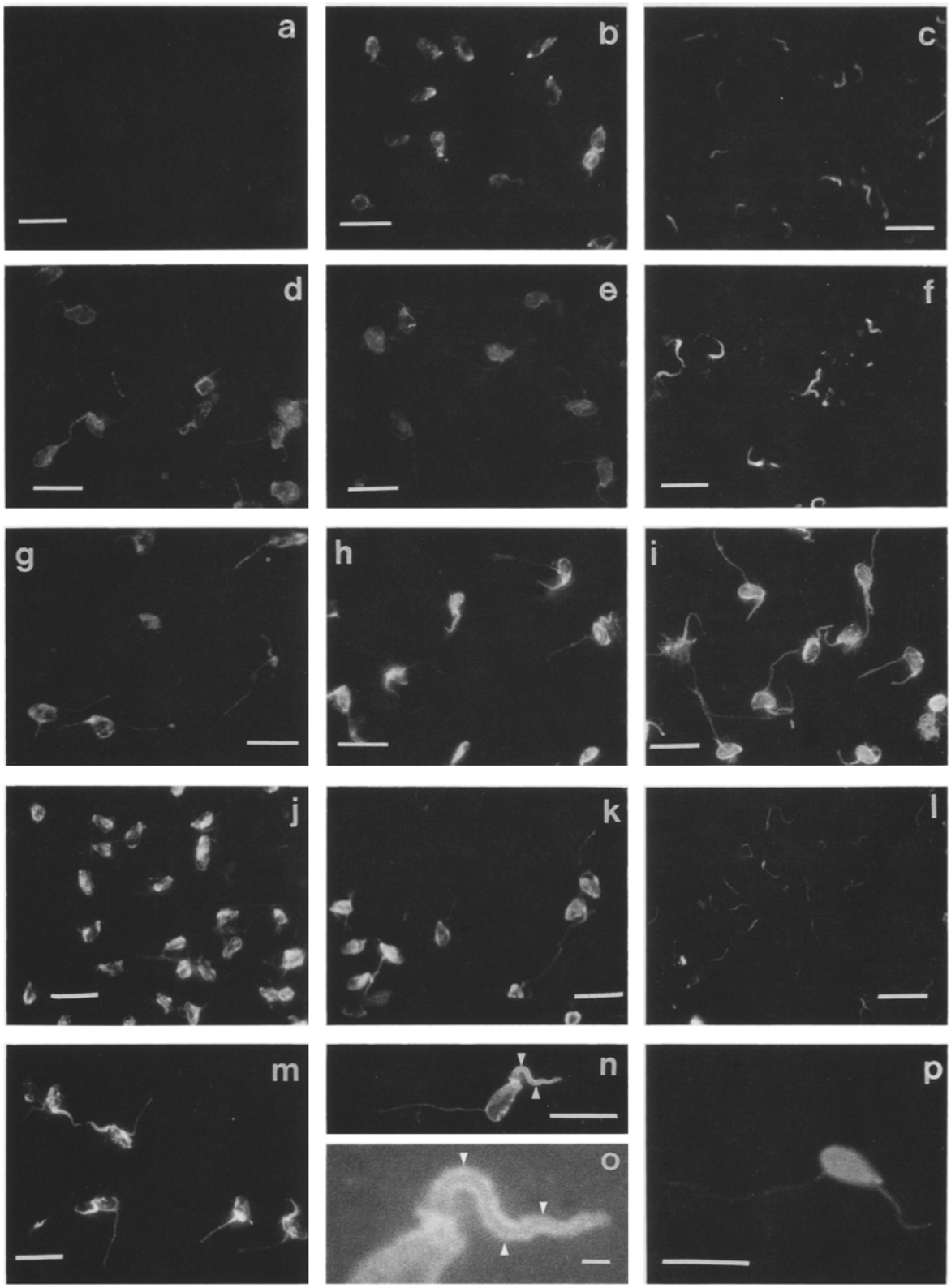

Fig. 1a–p. Indirect immunofluorescence of *Fucus serratus* sperm labelled with MAbs. **a** Control, **b** FS1, **c** FS2, **d** FS3, **e** FS4, **f** FS5, **g** FS6, **h** FS7, **i** FS8, **j** FS9, **k** FS10, **l** FS11, **m** FS12; **n** and **o** FS7 showing 'halo' effect on anterior flagellum; **p** FS4 showing non-halo on anterior flagellum. Magnification **a–m** ×800, **n** ×1100, **p** ×1400, bar=10 μm; **o** ×4400, bar=1 μm. *Arrows* highlight the 'halo'

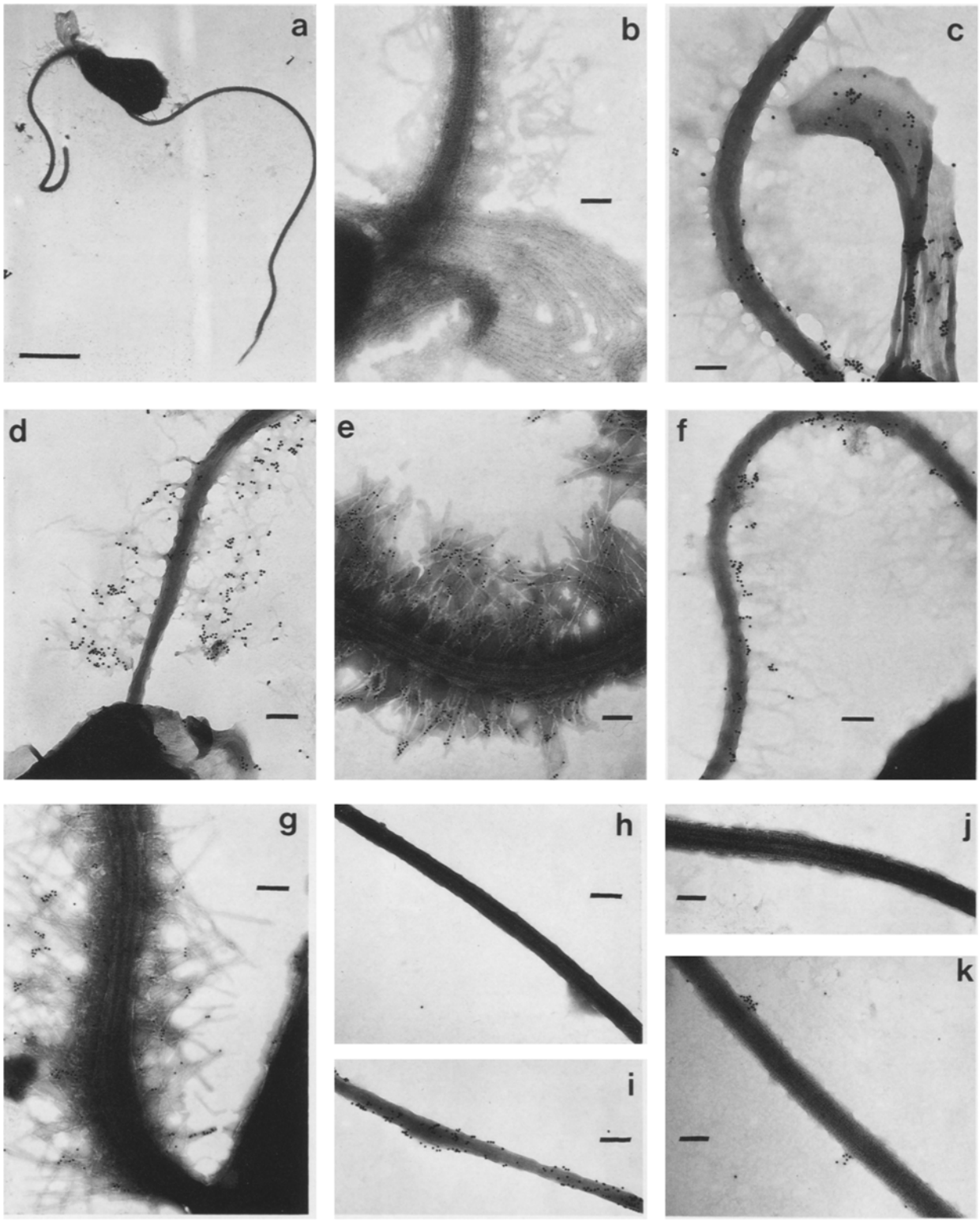

Fig. 2a–k. Immunogold labelling of negatively stained *Fucus seratus* sperm observed by EM. **a** Whole sperm cell, **b–c** anterior flagellum and proboscis, **d–g** anterior flagellum; **a**, **b** control, **c** FS4, **d** FS7, **e** FS2, **f** FS11, **g** FS1. **h–k** Posterior flagellum; **h** control, **i** FS4, **j** FS1, **k** FS9. Magnification: **a** ×3375, bar = 3 μm; **b–k** ×25500, bar = 0.2 μm

label the perimeter of the anterior flagellum giving a 'halo' effect, as exemplified by FS7 in Fig. 1n, o, whereas FS3, FS4, FS6, FS8, FS9, FS10 and FS11 do not form a halo in labelling the anterior flagellum, as exemplified by FS4 in Fig. 1p. In addition, it was not possible to ascertain by IIF whether MAbs bound to the proboscis on the body of the sperm. These aspects were investigated further using immunogold labelling.

Immunogold labelling of sperm flagella and proboscis. The mastigonemes (hairs), borne on the anterior flagellum, can be resolved on negatively stained sperm under the EM (Fig. 2a, b). The smooth posterior flagellum lacks such hairs (Fig. 2a, h). The possibility that the 'halo' results from the MAb binding to the mastigonemes rather than the flagellar shaft was confirmed by immunogold labelling, and the binding patterns observed are illustrated by typical examples in Fig. 2. The MAbs producing the 'halo' pattern (FS2, FS5, FS7, FS12) primarily or exclusively label the mastigonemes (Fig. 2d, e) whereas those that do not form a halo (FS3, FS4, FS6, FS8, FS9, FS10, FS11) primarily label the shaft of the flagellum (Fig. 2c, f). Interestingly, FS1, which by IIF appears to label the body exclusively, also binds the mastigonemes (Fig. 2g) indicating that EM-immunogold labelling is more sensitive than IIF. Binding of MAbs to the posterior flagellum was also investigated using immunogold labelling and typical examples are illustrated in Fig. 2h–k. As expected from the IIF studies FS3, FS4, FS6, FS8, FS9 and FS10 bind to the posterior flagellum with varying intensity, though FS1 does not bind (Fig. 2h–k).

The proboscis can also be resolved by negative staining (Fig. 2a, b) and all the MAbs which bound heavily to the sperm body as assessed by IIF (FS3, FS4, FS6, FS7, FS8, FS9, FS10, FS12) exhibited labelling of the proboscis (e.g. FS4, Fig. 2c). FS2 and FS5 also showed some weak labelling of the proboscis (not shown).

Binding of MAbs to egg antigens. Anti-sperm antibodies were tested for cross-reactivity with egg membranes by ELISA. A MAb raised against egg antigens (11.3A9.C2; data not shown) which binds strongly to eggs, and which cross-reacts with sperm membranes, was used to validate the assay. In the initial screens of hybridoma culture supernatants about half of the anti-sperm antibodies were found to bind to egg antigens. Of the twelve cell lines cloned (FS1–12), four MAbs bind to egg membranes as well as to sperm (Table 1) and they include the two MAbs which bind almost exclusively to the mastigonemes on the sperm (FS2, FS5) and two which bind to the whole of the sperm surface (FS4, FS9). The majority of the MAbs, however, bind to gamete-specific antigens present on the sperm cell surface but absent from the egg.

Table 2. Comparative binding of MAbs FS1–FS12 to sperm of *Fucus serratus*, *Fucus vesiculosus* and *Ascophyllum nodosum* as determined by indirect immunofluorescence. Fixed sperm mounted on glass slides were incubated with MAb followed by FITC-RAMIG and observed by epi-fluorescence microscopy. Intensity of fluorescence is recorded as follows: − absent; +− very weak; + weak; ++ intermediate; +++ strong

MAb	*F. serratus*	*F. vesiculosus*	*A. nodosum*
FS1	++	++	++
FS2	++	+++	−
FS3	++	++	+−
FS4	+++	++	++
FS5	+++	+++	+−
FS6	++	++	+−
FS7	+++	++	+
FS8	++	+	−
FS9	+++	+++	+++
FS10	+++	+	+−
FS11	++	+	+−
FS12	++	+	−

Preferential binding of MAbs to sperm of different species. Immunofluorescence was used to compare the pattern and intensity of labelling of sperm of *F. serratus*, *F. vesiculosus* and *A. nodosum* by the MAbs. FS10 labels sperm of *F. serratus* much more intensely than sperm of *F. vesiculosus* (Table 2) but all the other MAbs label the sperm of the two *Fucus* species with approximately the same intensities (Table 2). FS10 labels sperm of *A. nodosum* even less intensely than that of *F. vesiculosus* as do FS2, FS3, FS5, FS6, FS7, FS8, FS11 and FS12 (Table 2). In those cases where MAbs cross-reacted with species other then *F. serratus* the binding pattern exhibited was the same as that for *F. serratus*. There is no correlation between the region-preferential binding pattern and species- or genus-preferential binding.

Discussion

Monoclonal antibodies (MAbs) have been raised against components of the surface of *Fucus serratus* sperm in order to study the organisation of membrane antigens. Localisation studies, using IIF and EM-immunogold techniques, have shown that on the *Fucus* sperm cell some surface antigens are highly concentrated in particular regions whereas

others are more generally distributed over the cell surface. The anterior flagellum is clearly a specialised region of the cell as it bears mastigonemes, and three MAbs bind preferentially to this region; FS11 specifically labels the flagellar membrane, while FS2 and FS5 bind to the mastigonemes, though they also cross-react very weakly with the sperm body. This is in accordance with previous work on gametes of the unicellular, green alga *Chlamydomonas eugametos* which has also shown that flagella bearing mastigonemes have a distinct complement of surface molecules as compared with the rest of the cell (Musgrave et al. 1986). The mastigonemes on the *Fucus* sperm are themselves composed of three sections (Bouck 1969), but at the level of resolution used for the immunogold studies here, it is not possible to assign the MAb binding to any particular section.

The surface of the sperm body is another region which shows some molecular specialisation since it is predominantly labelled by FS1; weak labelling of mastigonemes with FS1 is only apparent when examined at the EM level. Though a number of MAbs bound to the proboscis and posterior flagellum, no MAbs were obtained which revealed antigens unique to these regions. Other antigens are less restricted in their distribution since several of the MAbs obtained label the entire sperm surface. However, though FS7 and FS12 bind to antigens which are distributed over the entire cell surface, they are highly concentrated towards the anterior region of the cell. Further, several MAbs (FS 3, FS 4, FS 6, FS 8, FS 9, FS 10) bind preferentially to the body of the sperm though they also label both flagella. Thus it appears that *Fucus* sperm can maintain antigens in distinct regions (anterior flagellum, mastigonemes, sperm body) while at the same time maintaining antigens at different concentrations over the whole surface of the sperm.

Spatial restriction of specific molecules to particular regions, and polarisation of the cell surface, is known to be common in animal cells (Gumbiner and Louvard 1985). These results with *Fucus* sperm clearly show that the surface antigens of a highly differentiated plant cell can also be organised in a non-uniform manner over the cell. This contrasts with recent work with sperm of the fern *Pteridium aquilinum,* which showed that two MAbs to cell surface antigens bound uniformly over the cell, though the possibility that other antigens are organised in a non-uniform manner has not been precluded (Marc et al. 1988). With mammalian sperm, freeze-fracture studies demonstrated that the cell surface is comprised of topographically distinct regions called domains (e.g. anterior head region, posterior head region, tail; Holt 1985). Monoclonal antibodies against the sperm surface have shown that such domains contain unique antigens and for example, in boar sperm, Saxena et al. (1986) report that of fifty-eight MAbs raised to surface antigens, all bound to one of five discrete domains (e.g. head, tail, midpiece) each with distinct boundaries. Other work has shown that in some instances antigens can be shared between adjacent regions, though only rarely have MAbs been generated that reveal an antigen distributed over the entire cell surface (Primakoff and Myles 1983; Gaunt et al. 1983). By comparison, it seems that the restriction of antigens to particular regions on the surface of *Fucus* sperm is less apparent than for mammalian sperm (only the FS11 antigen is totally restricted to the anterior flagellum membrane), whereas polarisation or concentration of antigens which are generally expressed over the sperm surface is more apparent.

The complex organisation of cell surface components in *Fucus* sperm has implications for cell differentiation and cell surface assembly and also for the maintenance of antigens in particular locations on the cell surface. Components must be targeted to their correct destination on the cell surface during cell surface development and turnover. The only work so far on *Fucus* sperm which has addressed this kind of problem is a study by Bouck (1969) on flagellar ontogeny, in which the fate of presumptive mastigonemes was traced from within the perinuclear space to membrane-limited cytoplasmic sacs and then to the surface of the otherwise mature anterior flagellum. The use of the MAbs, in particular FS2 and FS5, which are directed against mastigoneme antigens, and FS11, which binds to the membrane of the anterior flagellum, should prove useful in extending the studies of Bouck (1969) and should allow an analysis of the differentiation and assembly of the sperm during ontogeny within the antheridia.

The mechanisms by which the spatial organisation of *Fucus* sperm surface antigens is maintained are not known but it is possible that they are similar to those operating in animals cells, where domains are maintained by barriers to free diffusion, in the plane of the membrane, or by cytoplasmic anchors to the surface macromolecules (Gumbiner and Louvard 1985). Such barriers exist on the surface of mammalian sperm cells (Cowan et al. 1987), and in gametes of the green alga *Chlamydomonas eugametos* there is a functional barrier to free diffusion of surface macromolecules, located at the the flagellum-body transition zone (Musgrave et al. 1986).

The majority of the MAbs to the *Fucus* sperm do not bind to eggs (FS1, FS3, FS6, FS7, FS8, FS10, FS11 and FS12), indicating clearly that the sperm and egg cell surfaces differ. This confirms earlier reports (Bolwell et al. 1979, 1980; Catt et al. 1983; Vithanage et al. 1983) and shows that the concept of 'differentiation antigens' (Williams 1980) is also applicable to specialised plant cells. Such MAbs could be of particular value with respect to studies of sperm-egg recognition because evidence obtained indicates that this is heterophilic (Bolwell et al. 1979, 1980; Catt et al. 1983; Vithanage et al. 1983). However, not all sperm-specific antigens will necessarily be involved in egg recognition since sperm cells possess other specific functions (e.g. motility). In this context it is worth noting that antigens which are localised to particular regions in mammalian sperm have specific functions. For example, MAbs that inhibit fertilisation by preventing sperm penetration of the zona pellucida bind specifically to antigens in the 'anterior head' domain which overlies the acrosome (Saling and Lakoski 1985; Saling 1986), whereas those that inhibit sperm-egg plasma-membrane fusion bind the 'posterior head' domain (Saling et al. 1985; Primakoff et al. 1987). Whether the spatial restriction of antigens on the *Fucus* sperm surface is of parallel significance to that in mammalian sperm remains to be seen. Although there is no acrosome reaction during fertilisation in *Fucus* it is still possible that different surface antigens, with different surface locations, are involved in mediating attachment, recognition and fusion.

At a different level of specificity only one MAb (FS10) binds preferentially to sperm of *F. serratus* as compared with that of *F. vesiculosus,* though nine (FS2, FS3, FS5, FS6, FS7, FS8, FS10, FS11, FS12) bound preferentially to sperm of these species when compared with that of *A. nodosum.* These results reflect the degree of homology between the three species, showing that a number of antigenic determinants are highly conserved whereas others show variation. Such MAbs may be of use in studies of recognition, since sperm-egg recognition is species-specific, and also of value in clarifying the complex phylogenetic and taxonomic relationships of the fucoid algae.

Taken as a whole, the binding characteristics of the MAbs FS1–12 indicate the possibility that some may be binding the same epitope (e.g. FS2 and FS5; FS7 and FS12; FS3, FS6 and FS8). Competition binding assays indicate that this may in fact be the case though more detailed molecular analysis is revealing a more complicated relationship, possibly involving overlapping epitopes, and this will be the subject of a further report. In addition, investigations into possible functional effects of the MAbs on sperm motility, chemotaxis and fertilisation are in progress.

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