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The *Drosophila* position-specific antigens are a family of cell surface glycoprotein complexes

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Position-specific (PS)1 and PS2 monoclonal antibodies bind non-uniformly to the mature wing imaginal disc of *Drosophila* with respect to the boundary separating the dorsal and ventral developmental compartments. PS1 antibodies preferentially recognize dorsal cells, PS2 antibodies ventral cells. Antibodies of the two classes extract distinct sets of glycoproteins from an imaginal disc lysate. PS3 antibodies bind to both dorsal and ventral disc cells and extract both PS1 and PS2 glycoprotein sets together with an additional component. We show that the PS antigens are related multimeric glycoprotein complexes on the cell surface. PS3 antibodies recognize a glycoprotein present in all complexes, while PS1 and PS2 antibodies recognize unique components of their own complexes. Spatial and temporal correlations suggest the molecules may have a function in development.

Key words: cell surface antigens/compartments/*Drosophila* development/glycoproteins

Introduction

During *Drosophila* embryonic and larval development, the cells which form the imaginal discs become progressively specified in terms of the adult structures they will produce (Hadorn, 1965; for review, see Bryant, 1978). Regional organization of the discs into compartments also occurs (Garcia-Bellido *et al.*, 1973) and morphogenetic processes produce the discrete discs and mould the characteristic morphology of each disc type (for review, see Poodry, 1980). For morphogenesis, regional differences in the rate and direction of growth, shape of cells, and so on, must be set up. Several common enzymes show activities which are localized to different areas of discs at different stages of development (Sprey *et al.*, 1982); this suggests that general metabolic activity does vary regionally. While nothing is known of the mechanisms causing these differences in cell behaviour in the disc, a number of observations have led to the proposal that compartments, or their boundaries, may function to provide an overall control of size and shape (Lawrence and Morata, 1976). Despite the evidence of a high level of organization, imaginal discs show few signs of overt differentiation at the cellular level. For the most part, disc epithelial cells appear identical and, although early studies of disc major proteins using 2-D gel electrophoresis had suggested interdisc differences (Rodgers and Shearn, 1977), later studies revealed few differences, either between different disc types (e.g., wing and leg) or from region to region within a particular disc (Greenberg and Adler, 1982; Ghysen *et al.*, 1982). Cell surface antigens, defined by monoclonal antibodies, rarely show other than a homogeneous distribution on disc epithelia (D.L.Brower, R.A.H.White and M.Wilcox, unpublished). The relative homogeneity of the proteins expressed in discs means that molecules which show a restriction to specific populations of cells within the disc epithelium might reasonably be regarded as putative components of processes involved in the organization and determination of discs. This expectation would be strengthened if the distribution of the molecules correlated with known developmental events within the disc.

We have isolated a set of monoclonal antibodies which reveal the presence of such regionally restricted antigens. The distribution of these antigens within the mature wing imaginal disc correlates with the compartmentalization separating the dorsal and ventral cell populations of the disc. One class, position-specific (PS)1 antibodies, bind preferentially to dorsal cells (Wilcox *et al.*, 1981) while PS2 antibodies show a complementary ventral preference (Brower *et al.*, 1984). Antibodies of the two classes extract related but different cell surface antigens from disc lysates, each comprising a number of glycoprotein components (Brower *et al.*, 1984). A third class of antibody, PS3, binds to both dorsal and ventral cells and extracts both PS1 and PS2 antigens together with an additional component. This paper shows that these antigens are multimeric glycoprotein complexes expressed on the surface of the cells. For these studies we have used cells of one of a number of established *Drosophila* cell lines which carry the PS antigens. These cells provide a more convenient source of antigens than imaginal discs.

Results

In these studies we have used five PS1 monoclonal antibodies, one PS2 antibody and seven PS3 antibodies. Details of these are given in Materials and methods.

PS antibodies bind to cells of a number of established cell lines

Cells of 11 *Drosophila* cell lines were tested, in an indirect radioimmunoassay, for their ability to bind antibodies representative of the three classes. All the antibodies within each class showed approximately the same level of binding. Composite results are shown in Table I. Emal[1] cells bound the highest levels of each antibody. Cells of three lines, D1, ECS and EAdh, showed only low levels of binding of any PS antibody. Of the other seven lines, cells of only three bound significant amounts of the PS1 antibody but most were recognized to a variable extent by PS2 and PS3 antibodies. In all cases, the PS3 antibody showed the highest level of binding, always 50% or more of the total PS antibody binding. The ratio of PS3 antibody bound to the sum of PS1 and PS2 antibodies bound ranged from 1.5 to 5.0 indicating that the amount of the PS3 antigen on the cell surface may vary independently of the levels of the other two antigens.

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Table I. Binding of PS antibodies to cells of a number of *Drosophila* cell lines

Line	Background binding	Level of antibody binding			Ratio PS3/(PS1 + PS2)
		PS1 (%)[a]	PS2	PS3	
DM1	7	6	13	95	5.0
DM3	5	3	10	63	4.9
D1	5	8	4	11	0.9
ECS	3	2	4	11	1.8
EFMI	5	5	17	44	2.0
EOR	6	3	11	32	2.3
EAdh	3	2	6	16	2.0
Emal[1]	6	32	35	100	1.5
Er[1]	7	12	14	61	2.3
Er[36]	5	2	13	38	2.5
K_c	4	25	.16	61	1.5

Binding of PS antibodies to cells of the different cell lines was measured in an indirect radioimmunoassay as described in Materials and methods.
[a]Binding is expressed as a percentage of the binding of PS3 antibodies to Emal[1] cells (= 100%); this is the highest level of binding found. Levels of background binding (column 2) were subtracted to give the antibody binding values given in columns 3–5.

The PS antibodies extract very similar sets of glycoproteins from lysates of imaginal discs and of Emal[1] cells

We have reported (Brower *et al.*, 1984) that the antigens extracted by antibodies of the three classes from Emal[1] lysates, prepared by lysing the cells in the non-ionic detergent Nonidet P-40, each contained a number of glycoprotein components which could be resolved by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Figure 1 (lanes b–d) shows the components of the PS1, PS2 and PS3 antigens. Each antigen contains a gp110 component (which is sometimes resolved into two bands, see lane i for example) and two or more higher mol. wt. glycoproteins, while the PS3 set includes another component, gp92, usually present as a doublet. A further component with a mol. wt. of ~100 kd (marked by an asterisk in Figure 1) appears to be a breakdown product of gp110 (see later section). Although we cannot exclude the possibility that one or more of these proteins is a degradation artefact, all were consistently obtained from cell lysates. However, quantitative differences were sometimes observed, and occasionally other bands were seen which may be related to one or other of the major components (e.g., a PS2 glycoprotein, gp130, lying just above gp125 in lane c). Additional bands found only rarely have been ignored so far.

It is striking that the PS3 antigen appears to contain all the PS1 and PS2 antigen components, in addition to the unique gp92. Experiments in which lysates were sequentially extracted with antibodies of the different PS classes confirmed that the antigens were related (Brower *et al.*, 1984). Thus, prior extraction by any of a number of PS3 antibodies left no antigens for subsequent extraction by PS1 or PS2 antibodies. PS1 and PS2 antibodies, in contrast, extracted discrete subsets of the PS3 glycoprotein set (Figure 1 lanes e–f; see also Brower *et al.*, 1984) leaving behind a residual PS3 set (lane g). Further, all PS1 antibodies extract the same antigen: prior extraction with one such antibody left no antigen for subsequent extraction by any other antibody of the class (data not shown).

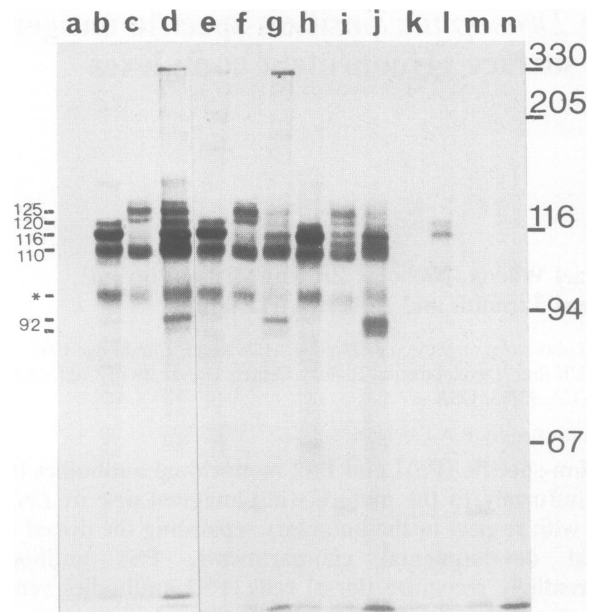

Fig. 1. The components of the three PS antigens. SDS-PAGE on 8% gels of antigens extracted from cell and imaginal disc lysates by PS antibodies covalently attached to Affi-Gel. The methods are outlined in Materials and methods. Major glycoprotein (gp) components are indicated with their mol. wt. values. **Lanes a** and **k**: controls (Emal[1] cell lysates extracted by deactivated Affi-Gel). **Lanes b–d**: PS1, PS2 and PS3 antigens, respectively, extracted from a lysate of Emal[1] cells. The PS3 antigen (**d**) contains (in addition to the unique gp92) components running identically to all of the PS1 and PS2 components. The remaining lanes show products extracted sequentially, first by PS1, then PS2 and finally PS3 antibodies from lysates of Emal[1] cells (**lanes e–g**), imaginal discs (**lanes h–j**) and D1 cells (**lanes l–n**). Each lysate was prepared from an equal volume of cells. After blotting to nitrocellulose, glycoproteins were detected by Con A/HRP staining (Hawkes, 1982). Marker proteins used were thyroglobulin (subunit 330 kd), myosin (205 kd), β-galactosidase (116 kd), phosphorylase B (94 kd) and bovine serum albumin (67 kd).

Figure 1 (lanes h–j) shows a sequential extraction of a total imaginal disc lysate (prepared from an equal volume of cells to that used for lanes e–g). Qualitatively the glycoprotein patterns are very similar to those of Emal[1] antigens (lanes e–g) although slight differences in the proportions of the different components are seen. The total amount per cell of each antigen extracted from the two lysates was also similar. These observations justify our use (for convenience) of the cell line antigens for the detailed analysis reported here.

Evidence suggests that the antigens extracted by the PS antibodies are indeed those visualized on the cell surface by immunofluorescence (Wilcox *et al.*, 1981; Brower *et al.*, 1984) and in radioimmunoassays (see preceding section). Sequential extraction of a lysate of cells of the D1 line (which showed little or no binding of PS antibodies, Table I) yielded only a trace of PS1 antigen components (Figure 1, lane l) and no detectable PS2 or PS3 components (lanes m and n). Further, we have shown that [^{125}I]antigens extracted from lysates of cells which had been ^{125}I-surface labelled using lactoperoxidase showed similar gel patterns to those revealed by lectin binding (Brower *et al.*, 1984, and see Figure 3).

The total antigens extracted by PS antibodies amounted to 0.22% of the TCA-precipitable ^{125}I-surface labelled material in a cell lysate. In a direct radioimmunoassay, using controlled dilution with unlabelled antibody, ~2 x 10^4 molecules of PS3 antibody bound per Emal[1] cell, again some 0.2% of the surface protein (since there are around 10^7 surface protein molecules on an average cell).

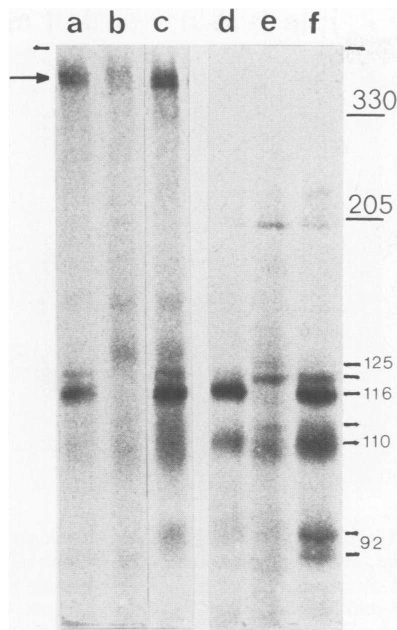

Fig. 2. Cross-linking of antigen components. PS antigens from cells which had been treated with DMS prior to lysis (see Materials and methods) were analyzed by SDS-PAGE on 8% gels (**lanes a – c**, PS1, PS2 and PS3 antigens, respectively; high mol. wt. material is arrowed). **Lanes d – f** show similar preparations from untreated cells. Glycoproteins were detected by Con A/HRP staining. Marker proteins are thyrogloblin (subunit 330 kd) and myosin (205 kd).

Surface cross-linking of the three antigen sets

The most obvious explanation for the relationship between the different PS antigens is that the antigenic determinant each antibody recognizes is present only on one of the glycoproteins, the other components being extracted because they are associated with this first molecule on the cell surface. To test this possibility, antigens were isolated from cells which had been treated with short-span (~10 Å) bifunctional cross-linking reagents.

In preliminary experiments, a non-reversible cross-linking reagent, dimethyl suberimidate (DMS; Davis and Stark, 1970) was used. Results are shown in Figure 2. From a lysate of DMS cross-linked cells, antibodies of each class pulled out new much higher mol. wt. components together with somewhat reduced amounts of the normal glycoproteins. The major complex bands, which often appeared as close-spaced doublets, had about the same mol. wt. around 450 kd (though, as cross-linked complexes run anomalously on SDS gels, this value is not reliable).

Sequential extraction of lysates in which the components had first been reversibly cross-linked, permitted the composition of each complex to be determined. The cells were first ^{125}I-surface labelled, and components then cross-linked using dithio bis(succinimidyl propionate) (DSP; Lomant and Fairbanks, 1976), which contains a dithiol bond labile to reducing agents. On SDS-PAGE, under non-reducing conditions, high mol. wt. bands were found in each antigen preparation (Figure 3, lanes 1 – 3), one in each at ~450 kd and a second weaker band, in the PS1 and PS3 extracts, at ~300 kd. Whether these weaker bands represent separate or partially cross-linked, complexes is unclear. The material in the various ^{125}I bands was detected after transfer to nitrocellulose, recovered by acetone precipitation, reduced and reanalyzed by SDS-PAGE. (This method proved more reliable than the alternative 2-D non-reducing/reducing SDS-PAGE).

Each of the two PS1 and the single PS2 high mol. wt. bands yielded the major components of the two sets. The PS1 complexes each contained gp116 and gp110 (Figure 3, lanes a – b), while the PS2 complex contained gp125 and gp110 (lane e). We were also able to detect the less abundant gp120 in these complexes by ConA/HRP staining (data not shown).

Both the residual PS3 complexes yielded, as major components, only gp116 and gp110 (lanes i – j). Some of this may be previously unextracted PS1 complex as the instability of the complexes precluded lengthy exhaustive extraction. No gp92 was found associated with either of the high mol. wt. bands.

Thus, the major glycoprotein components of each of the PS1 and PS2 antigens are closely associated on the cell surface. The situation is less clear for the residual PS3 antigen, of which gp92 is a major component. However, there are obvious reasons why cross-linking of even adjacent molecules may not occur. For reasons which are outlined later, we believe that gp92 must be associated with other components of a residual PS3 complex.

The lower mol. wt. bands in each case yielded predominantly one or other of the PS glycoproteins gp116 (lanes c and k), gp125 (lanes f and g), gp110 (lanes d, h, l and m) and gp92 (lane n). Under non-reducing conditions, most PS glycoproteins show altered mobilities – gp125, gp120 and gp116 run more slowly, at mol. wt. 140 – 160 kd (this could indicate the presence of S-S linked homopolymers), and gp110 faster (~100 kd). These changes are demonstrated more fully below. One additional component, at mol. wt. 100 kd, seen in lane m, is also found from time to time, in normal antigen preparations (see above). We present evidence later that it is related to gp110 and probably a breakdown product of it.

Antibodies of different PS classes recognize different molecules

A simple explanation for the specificity of antibodies of each class for their complex is that they recognize determinants on unique components of the complexes. Obvious candidates are gp116 and gp125 for the PS1 and PS2 antibody classes, respectively, and the apparently common component, gp110, for PS3 antibodies. We were not able to confirm this directly as none of the 20 antibodies tested which extract PS antigens will recognize any component on protein blots. However, strong indirect evidence exists to support the contention.

We find that the glycoprotein components of antigens bound to PS antibodies covalently attached to a solid support can be sequentially eluted by consecutive treatment of the columns with acidic and basic buffers. The sequence of release depends upon the class of antibody bound to the support. For four PS1 antibodies, gp116 and gp120 are preferentially retained by the antibody (an example is shown in Figure 4, lanes a – c). In contrast, gp125 is preferentially retained by the PS2 antibody (lanes d – f), in this case together with gp130 which is a variable component of the PS2 antigen. Most PS3 antibodies showed no preferential retention of any PS3 glycoprotein, but, in two cases, there is evidence for preferential retention of gp110 (lanes g – i).

We suggest that PS1 antibodies all recognize determinants on gp116 and gp120, PS2 antibodies recognize gp125 (and perhaps the infrequently seen gp130), and PS3 antibodies recognize a common gp110 component. In support of this,

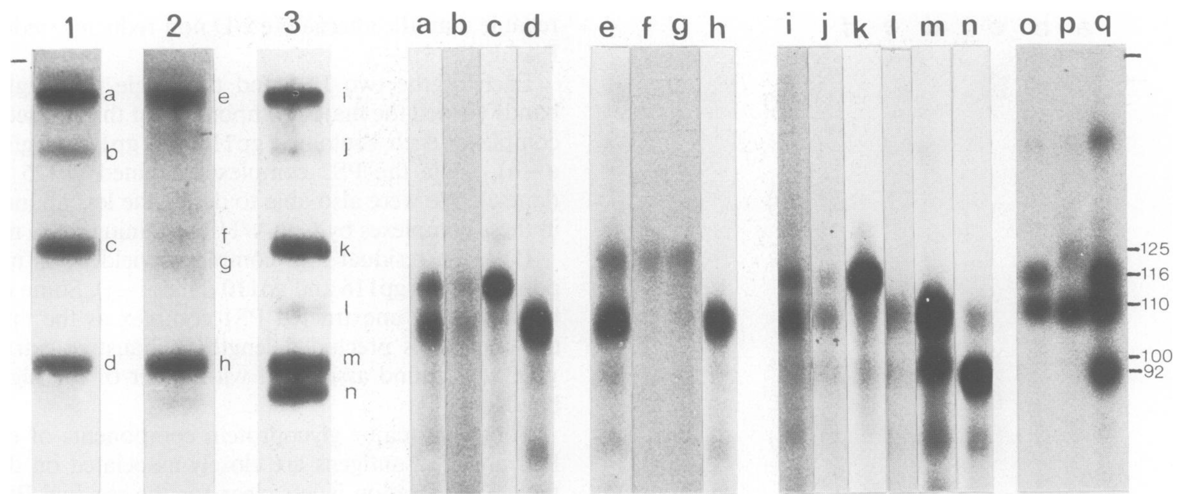

Fig. 3. Reversible cross-linking of antigen components. A lysate of cells, which had been surface ^{125}I-labelled and treated with the reversible cross-linking reagent DSP (see Materials and methods), was successively extracted by PS1, PS2 and PS3 antibodies, and the products run on an 8% non-reducing SDS-gel, transferred to nitrocellulose by protein blotting and detected by autoradiography (**lanes 1–3**). Strips containing bands a-n, indicated on these lanes, were cut out, soaked in 10 μl water containing 20 μg cytochrome c (as carrier protein), dried and dissolved in cold acetone (−20°C). After standing at −20°C overnight, precipitated protein was sedimented by centrifugation at 12 000 *g* for 10 min, the pellets rinsed with cold acetone, dried and re-dissolved in gel sample buffer containing 100 mM DTT. After heating for 5 min at 90°C, reduced products were re-run on an 8% reducing SDS-gel (**lanes a–n**). **Lanes o–q** show samples of the total cross-linked PS1, PS2 and PS3 antigens, respectively, reduced and run on the same gel.

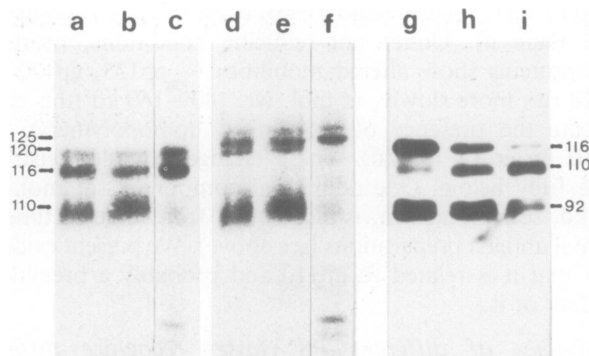

Fig. 4. Preferential retention of different antigen components by antibodies of the three PS classes. Antigens were extracted from lysates by antibody-Affi-Gel and the support beads washed and loaded into small columns as described in Materials and methods. Components were removed by successive elution with 2 x 1 column volumes of 100 mM citrate buffer pH 3.25, containing 1% octyl-β-D-glucopyranoside (OG) and 1 x 1 column volume of 50 mM diethylamine (DEA) pH 11.5. No antigen components remained on the column after these treatments. Columns were washed with two column volumes of 5 mM Tris-HCl pH 8.1 after the acid elution to adjust the pH. Products were analyzed by SDS-PAGE on 8% (**lanes a–f**) and 10% (**g–i**) gels. **Lanes a–c**: PS1 components eluted by citrate/OG (**a–b**) and DEA (**c**). **Lanes d–f**: PS2 components eluted by citrate/OG (**d–e**) and DEA (**f**). **Lanes g–i**: PS3 components eluted by citrate/OG (**g–h**) and DEA (**i**). PS1 and PS2 glycoproteins were detected by Con A/HRP staining of protein blots, PS3 glycoproteins by direct binding of [^{125}I]Con A in the gel (Koch and Smith, 1982).

we find that several PS3 antibodies, including those which retain gp110, will extract gp110 from a SDS-treated lysate (data not shown). However, no PS1 or PS2 antibody we have tested will extract any components from such a lysate. It seems likely therefore that, although individual glycoproteins carry the different antigenic determinants, antibody recognition in most cases requires association of these molecules with the other components of the complexes.

Relationships between the different antigens

Limited proteolysis of the various PS glycoproteins suggests that some may be related. For example, V8 protease digests of the higher mol. wt. components, gp125, gp120 and gp116, include several common fragments (Figure 5a and b). The presence of some fragments only in one or other digest, however, confirms the differences between these components inferred from their recognition only by PS1 or PS2 antibodies. We cannot resolve at present whether the three glycoproteins stem from different (though related) genes or from differential processing of the product of a single gene.

Similar studies of the other PS glycoproteins hint also at relationships between gp110 and gp100 and between the two gp92 components (data not shown). The pattern shown in Figure 5a also suggests that the rather broad gp110 (e.g., see Figure 1, lane d) may contain two or more closely related components, perhaps those which can often be resolved in PS2 antibody extracts (Figure 1, lane i). The proteolysis patterns reveal no obvious relationships between any of the gp125/120/116, gp110/100 or gp92 doublet sets (see Figure 5a and c, which is a subtilisin digest), suggesting that at least three different polypeptides are involved.

Other data support and extend these observations. A 2-D SDS-PAGE analysis, with non-reducing followed by reducing dimensions, has revealed that gp125, gp120 and gp116 behave very similarly; each is retarded in the non-reducing dimension, and to the same extent, so that their relative mobilities remain unchanged (Figure 6); in contrast, gp110 migrates more rapidly under non-reducing conditions while the gp92 components migrate identically in both dimensions.

Solid evidence links gp110 to gp100. The two are extracted together from SDS-treated lysates by several PS3 antibodies and they behave anomalously, in a similar way, on SDS-urea gels (Figure 7) and on non-reducing gels (data not shown). Finally, six monoclonal antibodies we have recently isolated recognize gp110 and gp100 (if it is present in the preparation) in protein blots of all PS antigens (Figure 8). Because of its very variable occurrence and build up in stored lysates, we believe that gp100 is a breakdown product of gp110.

The antibodies which recognize gp110 in protein blots cannot strictly be termed PS antibodies as they do not bind to imaginal discs or cultured cells, nor do they extract antigens from cell lysates. These antibodies do not bind to the PS3-

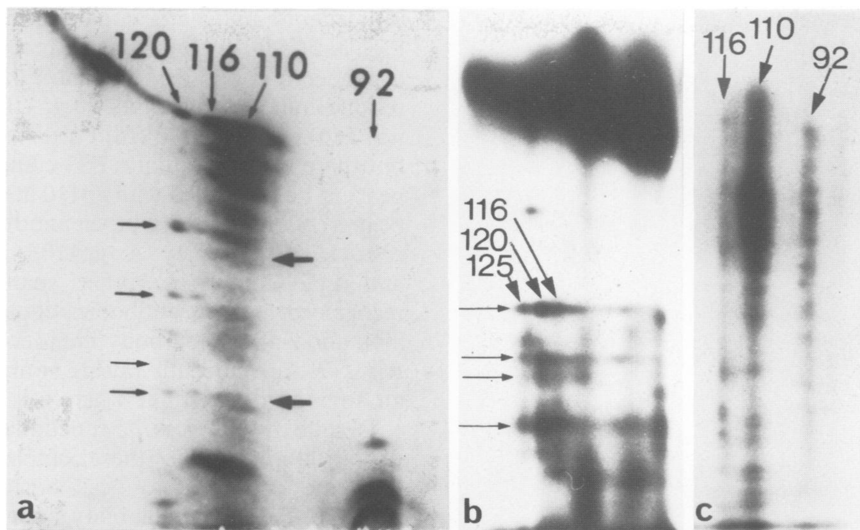

Fig. 5. Proteolysis of the components extracted by PS antibodies. Peptide mapping procedures were adopted from Bordier and Crettol-Järvinin (1979). **(a)** PS3 antigens were isolated from [^{35}S]methionine-labelled discs (Brower *et al.*, 1984; procedure II) and separated by SDS-PAGE on 7% gels. Individual lanes were cut from the gel, equilibrated 15 min in 0.2 M Tris-HCl, pH 6.8, 1 mM EDTA, 0.2% SDS, 7.5 sucrose, and placed across the top of a 5–20% gradient slab. *Staphylococcus aureus* V8 protease (Miles) was diluted at a pre-determined concentration in 0.1 M Tris-HCl, pH 8.8, 0.1% SDS, 10% sucrose and 0.002% Bromophenol blue, and 0.1 ml of the solution was layered on the top of the gel. Electrophoresis in the second dimension was halted for 30 min in the stacking gel to allow digestion to take place. The gp120 and gp116 components share a number of proteolytic fragments (thin arrows). In contrast, gp110 and gp92 have unrelated digestion patterns. Gp110, which runs as a broad band in this gel system, yields fragments of two distinct mol. wts. in some regions (thick arrows). **(b)** Combined PS2 and PS3 antigens from ^{125}I-labelled Emal[1] cells. Procedure as for **(a)** with the following modifications: (i) antigen components were first separated on an 8% gel, (ii) the second dimension was a 17.5% non-gradient gel. The gp125, gp120 and gp116 components yield a number of similar proteolytic fragments (thin arrows). **(c)** Residual PS3 antigen from [^{35}S]methionine-labelled discs (Brower *et al.*, 1984; procedure I). Procedure as for **(b)** except that subtilisin (Sigma; protease VII) was used. The three components show no obvious similarities in their digestion patterns.

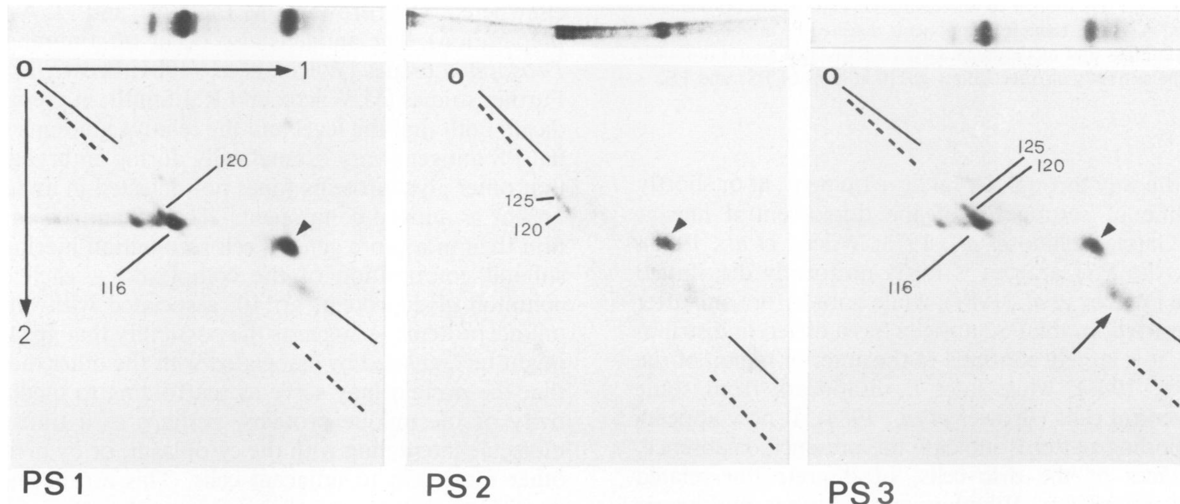

Fig. 6 The behaviour of antigen components on non-reducing gels. Samples of each antigen, prepared as described in Materials and methods, were run on 8% non-reducing SDS-gels, and lanes cut out and incubated in gel sample buffer containing 100 mM DTT for 2 h at room temperature. Each lane was then placed on top of a second 8% gel and proteins electrophoresed at 90° to the original direction of migration. Glycoproteins were detected by Con A/HRP after blotting to nitrocellulose. The upper photographs are 1-D non-reducing gel lanes to show the pattern for each antigen in the first dimension. The lower photographs show the 2-D patterns. O = original position of sample application. Solid lines show the position of the diagonal on which lie glycoproteins which migrate identically under both sets of conditions (the gp92 doublet, which shows this behaviour, is arrowed in PS3). Dotted lines indicate the position in each gel of a second line on which lie glycoproteins that migrate more slowly in the first dimension [gp120 and gp116 (PS1), gp125 and gp120 (PS2) and gp125, gp120 and gp116 (PS3)]. Note that these lines extrapolate to the origin, showing that these molecules are retarded to a similar extent in the non-reducing dimension. Above gp125 lies gp130 which is sometimes found in PS2 antigen preparations; this is retarded similarly. The common component gp110 migrates more rapidly under non-reducing conditions and so appears above the diagonal (arrowheads). Other products in the PS1 and PS3 gels which run slower than gp116 in the first dimension but identically to it in the second suggest the presence of higher homopolymers in the antigen preparations.

specific gp92 in blots, nor is gp92 preferentially retained by PS3 antibodies or extracted by them from dissociated complex mixtures. We conclude that gp92 is not closely related to gp110 and therefore must be associated with it in the residual PS3 antigen.

Discussion

The restriction of the PS1 and PS2 antigens, in a generally complementary way, to the dorsal and ventral cells of the wing imaginal disc only becomes apparent about three

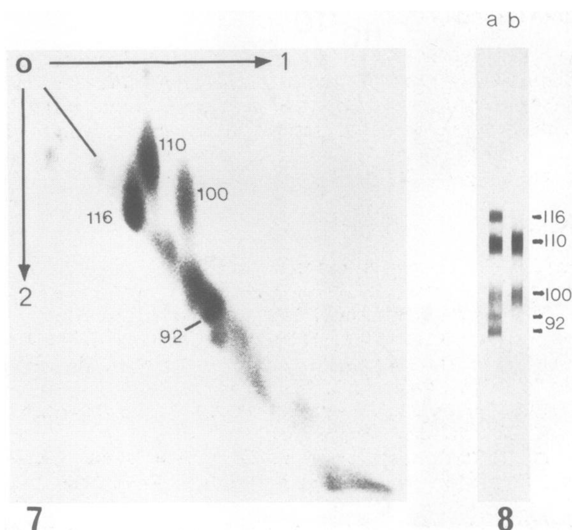

Fig. 7. Similar anomalous behaviour of gp110 and gp100 on a SDS-urea gel. Components of a residual PS3 antigen preparation, which had been stored at −20°C for some months, were fractionated on a 2-D SDS-gel, first under normal reducing conditions then in the presence of 4 M urea. O = original position of sample application. Glycoproteins were detected by Con A/HRP. Gp110 and gp100 are similarly retarded in the second dimensional and so run above the diagonal (indicated by a solid line). Other glycoproteins, gp116 and gp92, are unaffected by urea and are found on the diagonal.

Fig. 8. Monoclonal antibody which recognizes both gp110 and gp100 in protein blots. Residual PS3 antigen components were separated by SDS-PAGE on an 8% gel and blotted to nitrocellulose. **Lane a**: glycoproteins detected by Con A/HRP. **Lane b**: components detected by antibody DX.4C8 (see Materials and methods) and HRP-conjugated goat anti-mouse Ig (DAKO). The antibody also recognizes gp110 in blots of PS1 and PS2 antigens.

quarters of the way through larval development, at or shortly after the time of setting up of the dorso-ventral lineage restriction (Garcia-Bellido *et al.*, 1973; Wilcox *et al.*, 1981). Before this, the PS1 antigen is fairly uniformly distributed over the disc (Wilcox *et al.*, 1981), while both before and after its ventral restriction, the PS2 antigen has a different distribution: earlier, it is largely confined to the anterior region of the disc (Brower, 1984), while later it disappears from some peripheral ventral cells (Brower *et al.*, 1984). It now appears that these binding patterns indicate the presence or absence, on the surfaces of the disc cells, of discrete but related glycoprotein complexes. Whether one or other multimeric complex is exposed on the surface of a disc cell seems to depend on that cell's stage of development and on its position.

We do not know the basis of the differential expression of the antigens. The restricted binding patterns could reflect cell-specific expression of the unique components carrying the antigenic determinants for the different classes of antibody. However, PS1 and PS2 antibody recognition seems to require association of the different components of the complexes. Alternatively, then, the specificites could stem from assembly or disassembly of the complexes or even from some change in their orientation on the cell surface. This implies the existence of mediating molecules, which could be one or other of the glycoproteins we have described, or as yet unrecognized components.

The status of the 'residual' PS3 antigen, that remaining after removal of the PS1 and PS2 antigens, is unclear. That it is a separate antigen is implied by the fact that the level of PS3 antibody binding to a number of cell lines varies independently of those of PS1 and PS2 antibodies. Some of the residue could be uncomplexed gp110 because PS3 antibodies will extract free gp110 from a dissociated complex mixture, but there is also a unique PS3 component, gp92, which appears to be associated with gp110 in cells. This complex could be present on all cells or be yet another spatially or temporally restricted PS complex. As gp110 is also present in both PS1 and PS2 complexes, other spatially restricted antigens recognized by PS3 antibodies could not be located by examination of the antibody immunofluorecence binding patterns. We are currently raising antibodies to purified gp92 in an attempt to determine where it is found in the disc.

Despite the absence of a functional assay, the data allow us to conjecture a role for these complexes. We have shown that they are composed of cell surface glycoproteins; their detergent-dependent solubility both *in situ* and in a purified state (unpublished data) suggests that they are tightly bound, though laterally mobile (Brower *et al.*, 1984) membrane components. The different complexes are expressed in different places at different times in a number of imaginal discs (D.L. Brower, M.Piovant and L.A.Reger, in preparation), and the division lines between PS1 and PS2 antigen domains, and sharp differences in the level of a particular antigen, correlate most clearly with a number of lineage and morphological discontinuities. It is plausible therefore that these proteins could mediate intercellular interactions involved in disc organization and morphogenesis (this is discussed more fully elsewhere; D.L.Brower, M.Piovant and L.A.Reger, in preparation). The antigens also occur on a number of other *Drosophila* tissues (Wilcox *et al.*, 1981; Brower *et al.*, 1984). Further studies (M.Wilcox and R.J.Smith, in preparation) indicate both that the level and the relative amounts of individual PS antigens vary dramatically during embryogenesis and that other glycoproteins (ones not detected in imaginal discs) appear as antigen components. The PS antigens might function then in a more general cell recognition mechanism. The subunit composition of the complexes − each contains a common glycoprotein, gp110, associated with one or more unique proteins − suggests the possibility that gp110 function might be regulated by associated with the other molecules, or that the protein may serve as scaffolding to mediate the activity of the unique proteins, perhaps as a transmembrane molecule interacting with the cytoplasm, or by presenting the other molecules to adjacent cells. This arrangement would provide a subtle means of exposing on the cell surface a variety of different glycoprotein structures.

Materials and methods

Hybridomas and monoclonal antibodies

Immunization regimes and details of fusions yielding the hybridomas which secrete the monoclonal antibodies used in this study have been described (Wilcox *et al.*, 1981; Brower *et al.*, 1984). The antibodies of the various PS classes used were: PS1, DK.1A4, DT.1B12, DT.3D10, CF.5E5, DU.1G11; PS2, CF.2C7; PS3, DT.2H10, CF.6G11, DT.1C2, DT.3A10, DU.1F12, DX.2H4, DX.3C4. One other antibody used, DX.4C8, is representative of a class which recognize gp110 (and gp100) in protein blots but extract no antigens from a cell lysate. Sonicated *Drosophila melanogaster* imaginal discs were used as immunogens for fusions DK and CF, DK.1A4 immunoaffinity-purified antigen for fusion DT, and DT.2H10 immunoaffinity-purified antigens for fusions DU and DX. Hybridomas were grown as ascites in mice to provide large amounts of antibodies. Antibodies were purified by affinity chromatography on protein A-Sepharose (pA-S; Pharmacia). For binding studies, antibodies were ^{125}I-labelled using chloramine T.

Drosophila cell lines

Growth. Cell lines used were all derived from primary embryonic cell cultures: Dm1 and Dm3 (Schneider, 1972); D1 (Schneider and Blumenthal, 1978); Eadh, ECS, EFMI, Emal[1], EOR, Er[1] and Er[36] (Bernhard *et al.*, 1979); K_c (Echalier and Ohanessian, 1969). The lines were the gift of Dr. C. Bebbington. Cells were grown, at 25°C, as standing cultures in D22 medium (cf. Schneider and Blumenthal, 1978) containing 10% fetal calf serum (FCS) except for cells of lines ECS, EFMI and Er[36] which were grown in modified M3 medium (Shields and Sang, 1977) containing 10% FCS. At confluence (2×10^6 cells/ml), cells were freed from the surface of the flask by vigorous shaking (foam could be dispersed by the addition of a small drop of octanol) and pelleted by centrifugation (1500 r.p.m./5 min). Cells were used directly for radioimmunoassays or, if antigens were to be isolated, washed twice with ice-cold Dulbecco's phosphate buffered saline (DPBS). If radiolabelled antigens were required, cells were labelled at this stage either internally with [^{35}S]-methionine or on the surface with ^{125}I according to the procedures described earlier for imaginal discs (Brower *et al.*, 1984).

Radioimmunoassays. All incubations were carried out at room temperature in D22 medium containing 10% FCS. For measurement of PS antibody binding by indirect radioimmunoassay, 4×10^6 cells were first incubated, for 1 h, with the desired PS antibody in 0.2 ml medium (control experiments were performed to ensure that maximal levels of binding were achieved), then, after two washes with PBS/0.1% bovine serum albumin, with [^{125}I]rabbit anti-mouse Ig (10^5 c.p.m./0.2 ml medium) for 1 h. After two further washes, cells were suspended in PBS and counted in a gamma counter. In controls, antibody was omitted from the first incubation.

A direct radioimmunoassay was used to determine the number of antigen molecules/cell recognized by PS3 antibodies. Cells were incubated with a saturating level of [^{125}I]pA-S purified DT.2H10 antibody in the presence of increasing amounts of unlabelled DT.2H10 antibody. Control reactions contained a 100-fold excess of unlabelled antibody. The protein concentration of the unlabelled antibody preparation was determined by the method of Lowry *et al.* (1951).

Immunoaffinity isolation of PS antigens. Methods have already been described in detail (Brower *et al.*, 1984). Cells, or *D. melanogaster* imaginal discs, were lysed by treatment with the non-ionic detergent, Nonidet P-40 (NP-40), in the presence of protease inhibitors and, after pre-incubation with deactivated Affi-Gel 10 beads, antigens extracted by the desired antibody covalently attached to Affi-Gel 10. The beads were washed and transferred to small columns made from Eppendorf yellow tips plugged with glass wool. Antigens were eluted with an SDS-containing gel sample buffer, except for the experiments described in the penultimate section of the Results. Here, antigen components were removed sequentially by elution first with citrate buffer, pH 3.25, containing 1% octyl β-D-glucopyranoside (Calbiochem), then with diethylamine, pH 11.5 (see legend to Figure 4).

Cross-linking of proteins on intact cells

^{125}I-Labelled cells were treated either with the non-reversible bifunctional cross-linking reagent, DMS, or with the reversible cross-linking reagent, DSP.

DMS treatment. Washed cells were suspended in a buffer containing 50 mM bicine/20 mM $MgCl_2$, pH 8.5 containing 4 mg/ml DMS. After 1 h at room temperature, cells were pelleted, washed with PBS and lysed as described earlier.

DSP treatment. Solution of DSP, which is relatively insoluble in aqueous buffer, was achieved by dissolving 10 mg in 0.5 ml dimethyl formamide and adding 50 ml of PBS at room temperature. One volume of this solution was added to cells suspended in an equal volume of ice-cold PBS. After 10 min in ice, cells were pelleted and the treatment repeated. After pelleting, cells were washed once in cold PBS prior to lysis.

Gel electrophoresis, protein blotting and detection of antigen components

SDS-PAGE followed the method of Laemmli (1970). Thin (0.75 mm) gels were found to give much better resolution of the various glycoproteins. For non-reducing gels, samples were applied in a buffer containing 125 mM Tris-HCl/2% SDS/10% glycerol/0.05% bromophenol blue, pH 6.5. For reducing gels, samples included 5% 2-mercaptoethanol or 100 mM dithiothreitol (DTT).

After electrophoresis, labelled antigen components were visualized either by enhanced autoradiography (for ^{125}I; Laskey and Mills, 1977) or by fluorography (for [^{35}S]methionine; Bonner and Laskey, 1974). Unlabelled antigen components were visualized, after blotting to nitrocellulose, by the procedure of Hawkes (1982) except that FCS was replaced by polyvinylpyrrolidone 360. Blots were incubated successively with Con A (50 μg/ml) and with horseradish peroxidase (HRP, 50 μg/ml), and HRP activity detected in a staining reaction.

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