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Genetic localization of a follicle cell protein locus in *Drosophila melanogaster*

Katrin Lineruth, Andrew Lambertsson, and Mona Lindberg
Department of Genetics, University of Umeå, S-901 87 Umeå, Sweden

Summary. Three variant forms of a "novel" set of follicle cell proteins (Fc) were found when screening geographic wild-type strains of *Drosophila melanogaster* by SDS-polyacrylamide gel electrophoresis of ^{35}S-methionine labelled ovaries. These variant forms were used to establish *X* chromosomal linkage and for further genetic localization by both recombinant analysis and by cytogenetical mapping. A locus involved in the synthesis of Fc proteins was localized to the 7C1-9 region, i.e. very close to the singed locus (21.0 cM). The number of Fc proteins, their variation and possible function is discussed.

Introduction

During the later stages of oögenesis in *Drosophila melanogaster*, the follicle cells of the developing egg chamber are engaged in the developmentally regulated synthesis of several protein components of the egg. The vitellogenin polypeptides are synthesized in stages 8–10 (Brennan et al. 1982), the vitelline membrane proteins mainly in stages 9–10 and the chorion proteins in stages 11–14 (Petri et al. 1976). We have previously described three follicle cell proteins (Lineruth and Lambertsson 1985) which, although synthesized in stage 10, could not be classified as vitelline membrane proteins according to the criteria for amino acid content stated by Petri et al. (1976). The molecular weights of these proteins were estimated to be 92 K, 82 K and 76 K, respectively (Lineruth and Lambertsson 1985). We are tentatively calling them Fc proteins (Follicle cell proteins).

As a step towards understanding the relationship between the different Fc proteins, their function and the mechanisms controlling their synthesis, they were localized genetically. In this report we describe three variant sets of the Fc proteins, two of which were found when screening geographic wild-type strains. The Fc genes were localized both cytogenetically and by recombinant analysis to the 7C1-9 region on the *X* chromosome and in the vicinity of the singed locus. The number of Fc structural genes and the cause of variation is discussed.

Materials and methods

Drosophila stocks and culture conditions. All wild-type and marked strains were from the Drosophila Stock Center, Umeå, Sweden. The three wild-type strains that were used for further analyses were Hikone, Israel and Shahrinau. The genotypes of the marked strains are listed in Table 1. Flies were raised on a standard potato mash, yeast and agar substrate at 25° C.

In vitro labelling of ovaries. Incorporation of ^{35}S-methionine (Amersham, 37.0–48.1 TBq/mmol; 1,000–1,300 Ci/mmol) into five ovaries was at a final concentration of 37 MBq/ml in a 10 µl drop of MME (Fristrom et al. 1973). Incorporation was for 30 min at room temperature and the handling of the samples was according to the schedule described by Tissières et al. (1974).

Electrophoresis. Discontinuous SDS-polyacrylamide gel electrophoresis (180 × 180 × 1 mm) was performed as described by Laemmli (1970), using a modification of the apparatus used by Studier (1973). The 8% slab gels were run for 17 h at 80 V. Each sample contained approximately 150,000 cpm. Gels were treated for autoradiography with Amplify (Amersham), dried and exposed to Kodak XAR-5 film for 40–50 h at −80° C.

Results

In order to detect any electrophoretic variants of the Fc proteins, we screened wild-type strains from our collection of geographic strains on SDS-polyacrylamide gels. Two variants of the Fc proteins were found among 21 geographic strains and the electrophoretic mobilities of all three Fc proteins were simultaneously affected. Therefore, to simplify the following discussion, we will refer to the three Fc proteins as a set of proteins. The three sets of Fc proteins are henceforth called Fc1, Fc2 and Fc3, respectively. The

Table 1. Marked strains

C(1)DX, y f/f car; T(1; 2)sn^{+72d}; bw^{D}
C(1)DX, y f/sn^{36a}
C(1)DX, y w f/cm ct^{6} sn^{3}
C(1)DX, y w f/ct^{n} oc
C(1)DX, y w f/Df(1)ct^{J4}, ct^{J4} f; Dp(1; 3)sn^{13a}; Ki
C(1)DX, y w f/y^{2} sc w^{a} ec cv sn^{3}
Df(1)c52/FM6, y^{31d} sc^{8} dm B
Df(1)ct$^{268-42}$, y ct$^{268-42}$/FM4, y^{31d} sc^{8} dm B
Df(1)HA32/FM7, y^{31d} sc^{8} w^{a} sn^{X2} v^{Of} g^{4} B
Df(1)KA14, sn^{3} m/FM7, y^{31d} sc^{8} w^{a} sn^{X2} v^{Of} g^{4} B
Df(1)RA2/FM7, y^{31d} sc^{8} w^{a} sn^{X2} v^{Of} g^{4} B
Df(1)sn^{c128}, sn^{c128}/FM6, y^{31d} sc^{8} dm B
y ct^{6} v f l(1)su(f)^{ts67g}

Offprint requests to: K. Lineruth

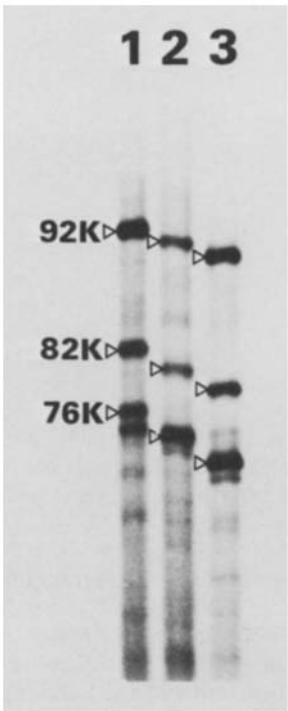

Fig. 1. Standard and variant forms of Fc proteins displayed on part of an SDS-polyacrylamide gel. The approximate molecular weights of the Fc1 proteins are shown; the approximate molecular weights of the Fc2 proteins are 90.5 K, 79 K and 74 K, respectively, and of the Fc3 proteins, 89 K, 77 K and 71.5 K, respectively. Lane 1, wild-type strain Hikone, displaying the standard Fc1 form; lane 2, wild-type strain Israel, displaying the variant Fc2 form; lane 3, wild-type strain Shahrinau, displaying the variant Fc3 form

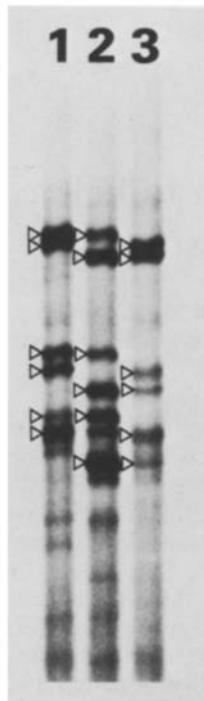

Fig. 2. Part of an SDS-polyacrylamide gel displaying the protein patterns of heterozygote F_1 females from crosses between wild-type strains. Lane 1, F_1 females from the cross Hikone × Israel; lane 2, F_1 females from the cross Hikone × Shahrinau; lane 3, F_1 females from the cross Israel × Shahrinau

Fc1 form is referred to as the standard form, found in nearly all marked strains analysed by us, and the most frequent among the wild-type strains. The Fc2 variant form was found in two geographic strains, Israel and Krasnodar, and in one marked strain. The Fc3 form was carried by four Russian strains, Alma-Ata, Frunze, Shahrinau and Tashkent. A possible case of intrastrain variation Fc1/Fc2 was found in the Swedish strain Karsnäs, but was not further analysed. Three strains were used for further analyses, Hikone (Fc1), Israel (Fc2) and Shahrinau (Fc3). The protein patterns of the three Fc forms are displayed in Fig. 1. The results from crosses between the different Fc variant forms revealed that the heterozygotes showed both the maternal and paternal forms of Fc proteins in approximately equal amounts (Fig. 2).

By a cross between *C(1)DX* females (females with *attached-X* chromosomes) carrying the Fc1 form and wild-type Fc2 males, we obtained F_1 females that were *C(1)DX* and heterozygous for the autosomes. These F_1 females displayed only the Fc1 form, indicating *X* chromosomal linkage. The next step was to cross a multiply marked strain that displayed the standard Fc1 form, to the Fc2 and Fc3 strains Israel and Shahrinau, respectively. For this purpose we chose a strain that contained $l(1)su(f)^{ts67g}$, the reasons for which will be discussed below. This strain was marked $y\ ct^6 v f\ l(1)su(f)^{ts67g}$. Heterozygous F_1 females were backcrossed to $y\ ct^6\ v\ f\ l(1)su(f)^{ts67g}$ males. Electrophoretic analysis of individual recombinant F_2 females indicated that the Fc genes were located near the cut locus (*ct* map position, 20.0 cM). This cross was repeated with respect

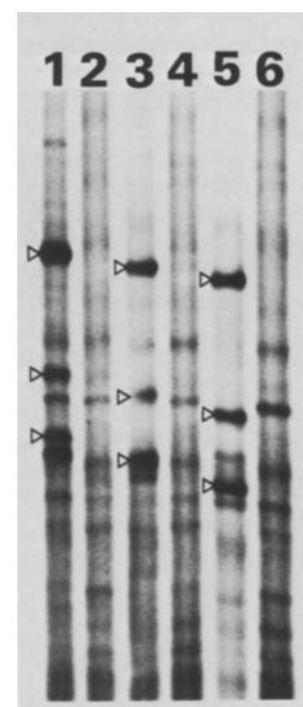

Fig. 3. Protein patterns of $l(1)su(f)^{ts67g}$ females at 25° C and after a shift to the restrictive temperature of 30° C. Part of an SDS-polyacrylamide gel is shown. Lane 1, standard $l(1)su(f)^{ts67g}$ females, displaying the Fc1 form, at 25° C; lane 2, same females as in lane 1, at 30° C; lane 3, variant $l(1)su(f)^{ts67g}$ females, displaying the Fc2 form, at 25° C; lane 4, same females as in lane 3, at 30° C; lane 5, variant $l(1)su(f)^{ts67g}$ females, displaying the Fc3 form, at 25° C; lane 6, same females as in lane 5, at 30° C

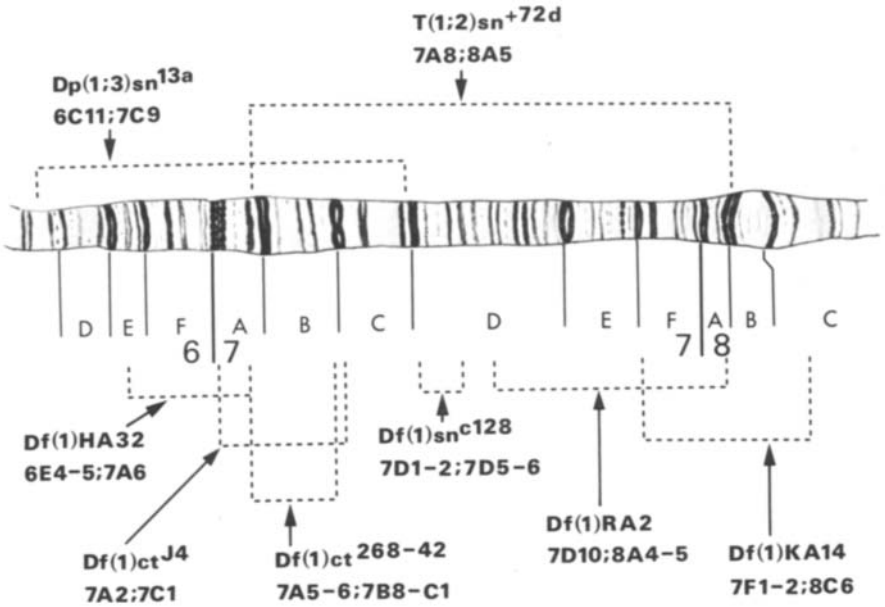

Fig. 4. Approximate cytological extent of the duplication, translocation and deficiencies used in the cytogenetic localization of the Fc genes. The polytene chromosome map is modified from Bridges (1938)

to recombinants between *ct* and *v*, and, by using the multiply marked y^2 *sc* w^a *ec cv* sn^3 chromosome in a similar recombinant analysis, the Fc genes could be localized to the cut – singed region (*sn* map position, 21.0 cM), probably limited distally at 19 cM and proximally at 22 cM.

Though both the Fc2 and Fc3 variant forms showed the same stage-specific labelling as the Fc1 standard form previously analysed (results not shown), we still wanted to confirm that the new variant forms were indeed variants of the Fc proteins. A particular reason for this was that the Fc2 protein 74 K appeared to comigrate with a protein observed in protein patterns displaying the Fc1 form (Fig. 1) and which did not belong to the Fc proteins. For this purpose we introduced $l(1)su(f)^{ts67g}$ into the strains Israel (Fc2) and Shahrinau (Fc3). When homozygous $l(1)su(f)^{ts67g}$ females are transferred to 30° C they become sterile and the synthesis of Fc proteins and several other follicle cell proteins ceases, while general protein synthesis appears to be unaffected. Analysis of the protein patterns after transfer to 30° C, revealed that the new variants showed a response to the temperature shift identical to that of the previously analysed Fc1 standard form (Fig. 3), thus confirming that the new variant forms really are Fc proteins. For results concerning the stage specificity of the Fc proteins and the effects of $l(1)su(f)^{ts67g}$ on ovarian protein synthesis after a shift to 30° C, see Lineruth and Lambertsson (1985).

For further localization of the Fc genes, a group of deficiency heterozygotes was obtained by crossing the Fc3 strain Shahrinau to six strains carrying *X* chromosome deficiencies in the 6E–8C region. These strains all displayed the Fc1 form and are listed in Table 1; their cytological extent is shown in Fig. 4. However, none of the deficiency heterozygotes that we analysed displayed the Fc3 form only, as would have been expected if the Fc genes were located in the deleted region. All six deficiency heterozygotes displayed both the Fc1 and Fc3 forms, indicating that the Fc genes are located either in the 7C1–7D1-2 or in the 7D5-6–7D10 region (Fig. 4). To confirm these results and, if possible, to discriminate between the two regions in question, we constructed females that were homozygous for the Fc3 form and in addition carried a chromosomal rearrangement involving the region of the *X* chromosome that we were interested in. These rearrangements were $T(1:2)sn^{+72d}$ (7A8–8A5) and $Dp(1:3)sn^{13a}$ (6C11–7C9). As the original strains carrying the chromosomal rearrangements displayed the Fc1 form, and as the females we had constructed were homozygous for the Fc3 form on the *X* chromosome, we expected that the females would show both the Fc1 and Fc3 forms if the rearranged segment of the *X* chromosome covered the Fc genes. As both types of females displayed both the Fc1 and Fc3 forms, we conclude that the Fc genes are located in the 7C1-9 region (Fig. 4).

The cytogenetical localization was confirmed by further recombinant analysis, using the marked chromosomes ct^n *oc* and *cm* ct^6 sn^3, which both displayed the Fc1 form. Shahrinau (Fc3) females were crossed to males carrying the marked *X* chromosome, and F_1 females were backcrossed to ct^n *oc* and *cm* ct^6 sn^3 males, respectively. Recombinant F_2 males were then individually crossed to Shahrinau females and the resulting F_3 females were analysed electrophoretically. Of 19 investigated recombinants in the *ct – oc* region, 3 recombinants between the *ct* and *Fc* locus were found, the other 16 were recombinants resulting from crossover between the *Fc* locus and *oc*. When using the *cm* ct^6 sn^3 chromosome, only 7 recombinants between the Fc locus and *sn* were found among 63 recombinants investigated in the *ct – sn* region, which indicates that the Fc locus maps in the vicinity of *sn*.

Because of the results from the recombination analysis, which placed the Fc locus near the singed locus, we tested the *sn* alleles sn^{X2} and sn^{36a}, which when homozygous cause female sterility (Bender 1960). The strains used are listed in Table 1; the sn^{X2} allele is included in the balanced chromosome of some of the marked strains. Crosses were made to obtain females that carried one of the *sn* alleles on one *X* chromosome and Fc1 or Fc3, respectively, on the other *X* chromosome. The protein patterns displayed by these F_1 females, eliminate the possibility that the *sn* alleles sn^{X2} and sn^{36a} might be involved in the synthesis of Fc proteins.

It should be noted that out of 113 cases where F_2 progeny from heterozygous F_1 females were analysed, no recombination between the Fc genes could be detected.

Discussion

When screening more than 20 wild-type strains, 3 variant forms of a set of follicle cell proteins were found. We have referred to these sets as Fc1, Fc2 and Fc3, respectively, as the variation in molecular weight involves all three proteins that compose a Fc protein set and as we have not observed any polymorphism for a single Fc protein. The simultaneous effect on all three Fc proteins, and the observation that the molecular weight differences between the proteins within a set appear to be more or less equal in the three variant forms are two striking observations. In the three possible crosses between the three wild-type strains we used for further analysis, the F_1 progeny displayed both the maternal and paternal forms of Fc proteins. By genetic analysis, a locus involved in the synthesis of the Fc proteins was located to the 7C1-9 region near the singed locus. There are, however, two points worth discussing: the actual number of Fc proteins and the possibility that we have mapped the gene for a modifying function.

If each set of Fc proteins consists of three different proteins, it implies that three independent mutations have occurred to make a variant set, which we find rather unlikely. We have recently obtained preliminary results, from in vitro cultured egg chambers, indicating that the largest Fc protein (92 K in the Fc1 form) is processed via the 82 K protein to the 76 K protein during the development of the egg chamber. This notion is supported by the time of detection of the three labelled Fc proteins: the 92 K and 82 K both at stage 10A and 10B and the 76 K at stage 10B only (Lineruth and Lambertsson 1985). The molecular weights refer to the standard Fc1 form. Experiments are in progress to elucidate this matter.

The fact that the F_1 heterozygotes from crosses between the different Fc forms display both the parental forms in all three possible crosses, suggests that the source of variation resides in the structural gene(s). This is very probable if there is only one Fc structural gene and the primary protein is processed as discussed above. On the other hand, we cannot rule out the possibility that we have mapped the gene for a modifying function, which is the source of the variation. However, this potential modifying function is not associated with the above mentioned processing of the Fc proteins. One explanation could be that a gene for a modifying function is located in the 7C1-9 region and the Fc structural gene(s) are located somewhere else in the genome. If so, we would expect the F_1 progeny to display a doubly modified form, different from both the parental forms, or if a null allele for the modifying function is involved, only a single form identical to one of the parental forms. As already mentioned, this is not the case, and regardless of the actual number of Fc proteins, this supposition is not consistent with our results.

As previously mentioned, the Fc proteins are stage specific and are synthesized by the follicle cells. We have recently obtained preliminary results indicating that the Fc proteins may be eggshell components. Furthermore, Komitopoulou et al. (1983) have characterized sex-linked female sterile mutants, with special attention to those that affect eggshell formation. Of these eggshell mutants, three map in the same cytological region as the Fc proteins. Two of these mutations interact with each other and the authors suggest that these genes interact in *cis* with respect to fertility. It is possible that one or more of the mutants analysed by Komitopoulou et al. (1983) are mutations affecting the Fc structural gene(s). The results from our recombinant analysis place the Fc locus in the vicinity of the singed locus, the cytological position of which is 7D1-2 (Lindsley and Grell 1968) and in a region that has previously been described as haplolethal, i.e. 7C5-9 (Lefevre and Johnson 1973). As one of the mutants analysed by Komitopoulou et al. (1983) seems to be located in this region, it supports further the assumption that at least one of these eggshell mutants may be involved in the production of the Fc proteins.

At the present time we favour the hypothesis that the largest Fc protein (92 K in the Fc1 form) is processed as the egg chamber develops and, consequently, that the variation is due to structurally altered forms of a single gene located in the 7C1-9 region. Therefore we suggest that the Fc2 and Fc3 variant forms each arose by a small deletion in the 92 K structural gene and that this deletion does not impair the function of the protein nor its further processing. However, mutation resulting in amino acid differences cannot be excluded. Experiments, including peptide mapping and analysis at the level of transcription, are now in progress to answer the questions of the number of Fc proteins and the role of these proteins in the development of the egg chamber.

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References

Bender HA (1960) Studies on the expression of various singed alleles in *Drosophila melanogaster*. Genetics 45:867–883

Brennan MD, Weiner AJ, Goralski TJ, Mahowald AP (1982) The follicle cells are a major site of vitellogenin synthesis in *Drosophila melanogaster*. Dev Biol 89:225–236

Bridges CB (1938) A revised map of the salivary gland X-chromosome of *Drosophila melanogaster*. J Heredity 29:11–13

Fristrom JW, Logan WR, Murphy C (1973) The synthetic and minimal requirements for evagination of imaginal discs of *Drosophila melanogaster* in vitro. Dev Biol 33:441–456

Komitopoulou K, Gans M, Margaritis LH, Kafatos FC, Masson M (1983) Isolation and characterization of sex-linked female-sterile mutants in *Drosophila melanogaster* with special attention to eggshell mutants. Genetics 105:897–920

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. Nature 227:680–685

Lefevre G, Johnson TK (1973) Evidence for a sex-linked haploinviable locus in the cut-singed region of *Drosophila melanogaster*. Genetics 74:633–645

Lindsley DL, Grell EH (1968) Genetic variations of *Drosophila melanogaster*. Carnegie Institution of Washington Publication No. 627

Lineruth K, Lambertsson A (1985) Stage specific synthesis of some follicle cell proteins in *Drosophila melanogaster*. Wilhelm Roux's Arch 194:436–439

Petri WH, Wyman AR, Kafatos FC (1976) Specific protein synthesis in cellular differentiation. III. The eggshell proteins of *Drosophila melanogaster* and their program of synthesis. Dev Biol 49:185–199

Studier FW (1973) Analysis of bacteriophage T7 early RNAs and proteins on slab gels. J Mol Biol 79:237–248

Tissières A, Mitchell HK, Tracy UM (1974) Protein synthesis in salivary glands of *Drosophila melanogaster:* Relation to chromosome puffs. J Mol Biol 84:389–398

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