The Drosophila Tissue Polarity Gene *inturned* Functions Prior to Wing Hair Morphogenesis in the Regulation of Hair Polarity and Number

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ABSTRACT

The adult cuticular wing of Drosophila is covered with an array of distally pointing hairs. Mutations in the *inturned* (*in*) gene result in both abnormal hair polarity (*i.e.*, hairs no longer point distally), and, in most cells forming more than one hair. We have isolated and characterized a collection of *in* alleles. Among this collection of alleles are a number of rearrangements that enable us to assign *in* to 77B3-5. Almost all of the *in* alleles, including putative null alleles, result in a stronger phenotype on the wing at 18° than 29°. The data argue that the *in*-dependent process is cold-sensitive. Temperature shift experiments with a hypomorphic allele show that this cold sensitivity can be relieved by several hours of incubation at the permissive temperature at a variety of times in the early pupae, but that this ability ends prior to the start of hair morphogenesis. One new allele showed a dramatic heat sensitivity. Temperature shift experiments with this allele revealed a very short temperature-sensitive period that is a few hours prior to the start of hair morphogenesis. That the temperature during hair morphogenesis is irrelevant for the phenotype of *in* is consistent with the hypothesis that the only role that *in* has in wing hair development is to regulate the initiation of hair morphogenesis.

THE adult cuticle of Drosophila is covered with a large number of polarized cuticular structures, which are typically aligned in parallel. For example, the wing is covered by an array of distally pointing hairs, giving the wing a "tissue polarity" (ADLER 1992). The adult cuticular hairs are formed in the pupae from microvillus-like prehairs (MITCHELL *et al.* 1981; FRISTROM *et al.* 1993; WONG and ADLER 1993) that are assembled at the distal-most vertex of each of the polygonally shaped pupal wing cells (WONG and ADLER 1993). The prehair being formed at the distal vertex appears to be essential for the development of distal polarity as mutations in six tissue polarity genes that alter adult hair polarity also alter the subcellular location for prehair formation (WONG and ADLER 1993).

Six tissue polarity genes have been placed into three phenotypic groups that also represent epistasis groups (WONG and ADLER 1993). The genetic data suggest that these genes comprise a genetic regulatory pathway which insures that prehair initiation occurs at the distal vertex. In a wild-type fly each pupal wing cell forms a single hair. The observation that most pupal wing cells in group 2 [*inturned* (*in*) and *fuzzy* (*fy*)] and 3 [*multiple wing hair* (*mwh*)] mutants form more than one hair suggests that this is a negative regulatory pathway that restricts prehair initiation to the distal vertex by inhibiting prehair initiation elsewhere. In a formal sense, the group 2 and 3 genes act as inhibitors of prehair initiation, although there is no reason to believe that these genes function directly in that way. For example, these genes could be serving as activators of a downstream inhibitor. None of these three genes has been well studied genetically. Because of our interest in tissue polarity and the regulation of the formation of cell extensions such as prehairs, we have undertaken a genetic analysis of the *in* gene.

We isolated and characterized a collection of 18 new *in* alleles. Included among these were a number of cytologically visible alterations that enabled us to determine the cytological location of *in* as being 77B3-5. All of the cytologically normal *in* mutations are viable as homozygotes or hemizygotes, which argues that *in* is a non-essential gene, as is the case for two other tissue polarity genes *fz* (*frizzled*) and *pk* (*prickle*) (GUBB and GARCIA-BELLIDO 1981; ADLER *et al.* 1987; HEITZLER *et al.* 1993). The phenotypes of most of the new *in* alleles were similar to that of in^1. Four alleles, however, had distinctly weaker phenotypes.

As part of the phenotypic characterization of the new *in* alleles, we examined adult wings from flies raised at either 18° or 29°. We found that all except one of the *in* alleles had a more severe phenotype at 18° than 29°. Since this was true of alleles that we suspect are null alleles, it seems likely that the *in*-dependent process is inherently cold-sensitive. Temperature shift experiments showed that the sensitive period for this phenotype started in the early pupae and ended prior to the first sign of hair formation. However, there was no particular time within this period when the permissive temperature was absolutely essential. Rather, incubation at the permissive temperature any time during the pupal period prior to prehair initiation provided at least

TABLE 1

List of *inturned* alleles

Allele	Reference[a]	Induced by[b]	Viable[c]	Phenotype[d]	Comment
1	LZ	S	V	S	
GL4b	TP	G	V	S	
GI1b	TP	G	V[e]	S	
GP4a	TP	G	V	S	
GN4a	TP	G	L	W	Heterochromatic inversion
ID22a	TP	G	L	W	Heterochromatic inversion
ID22b	TP	G	L	S	Heterochromatic inversion
IA11	TP	G	V-low	S	Heterochromatic inversion
IA41	TP	G	V	S	Complex rearrangement, adults short lived
IE41	TP	G	L	S	Deficiency
IA12	TP	G	L	S	Deficiency
HC31	TP	E	V	W	
HC62	TP	E	V	S	
HD31	TP	E	V	S	
HE61	TP	E	V	S	
II53	TP	E	V	W	Temperature-sensitive
IH52	TP	E	V	S	
IH56	TP	E	V	S	
CAH3a	TP	HD	V	S	Revertable

[a] TP, this paper; LZ, LINDSLEY and ZIMM, 1991.
[b] G, γ ray; E, EMS; HD, hybrid dysgenesis; S, spontaneous.
[c] Viable either as a homozygote or a hemizygote; V, viable; L, lethal.
[d] Strong or weak; S, strong; W, weak (see text).
[e] This stock was homozygous viable when originally isolated, but it no longer is. It presumably has picked up a lethal mutation.

partial rescue. One new *in* allele differed sharply from the others in that it displayed a very weak phenotype at 18°, but a fairly strong phenotype at 29°. Temperature shift experiments with this allele revealed a very short temperature-sensitive period that was a few hours prior to the first sign of prehair morphogenesis. Thus, the results from both sets of temperature shift experiments suggest that *in* functions prior to the actual morphogenesis of the prehair.

MATERIALS AND METHODS

Drosophila stocks: Marker mutations and balancer chromosomes are described in LINDSLEY and ZIMM (1992). Unless otherwise noted, all flies were grown at 25°. Several marker and Deficiency stocks were obtained from the Drosophila Stock Center at Indiana University.

Isolation of new *in* alleles: New *in* alleles were isolated in F1 mutant screens, where mutagenized male flies were crossed to in^1 females. The progeny of such crosses were screened for the abnormal bristle polarity phenotype of *in* (GUBB and GARCIA-BELLIDO 1981; LINDSLEY and ZIMM 1992). In most experiments the mutagenized chromosome was marked with *ri*, which is located just proximal to *in*. EMS, γ-ray and hybrid dysgenesis mutagenesis was performed as described for F_1 mutant hunts for new *fz* alleles carried out in this laboratory (ADLER *et al.* 1987). New mutant stocks were recovered in different EMS mutagenesis screens at a frequency that ranged from 0.05 to 0.035%. This frequency is consistent with the mutations being simple loss of function mutations.

Analysis of the *inturned* phenotype: Wings were mounted in euparal as described previously (ADLER *et al.* 1987) and examined under bright field optics. Unless noted otherwise, all data reported in detail are from *in* hemizygotes, where the individual allele is over $Df(3L)rdgC^{co2}$. Similar results were obtained in many experiments using alternative deficiencies.

As is the case for all of the known tissue polarity genes, the phenotype of *in* varies in severity across the wing (GUBB and GARCIA-BELLIDO 1981; ADLER *et al.* 1987; WONG and ADLER 1993). To compare the fraction of cells forming one, two or three hairs, we therefore always examined the same region of the wing. We scored 100 cells in a 20 × 5 cell rectangle located in the C cell on the dorsal surface of the wing. The long axis of the rectangle bordered the fourth vein and was centered on the posterior cross vein. This same region of the wing was scored in previous studies (WONG and ADLER 1993). We typically scored five individual wings for a genotype/temperature condition.

We determined the fraction of the dorsal surface of the wing displaying grossly abnormal hair polarity as described in ADLER *et al.* (1994). Briefly, all areas on the dorsal surface either where neighboring hairs did not share a common polarity or where hairs pointed 45° or more off from wild type were marked on a diagram of the wing. These diagrams were then scanned into a computer, and the fraction of the wing with abnormal polarity was determined using the NIH image program. As for other tissue polarity genes, the ventral surface of *in* wings typically shows a more severe phenotype (GUBB and GARCIA-BELLIDO 1981; ADLER *et al.* 1987), but we quantified the polarity phenotype on the dorsal surface because the dorsal hairs are larger, darker and easier to see and photograph.

Temperature shift experiments were carried out as described previously (ADLER *et al.* 1994). Briefly, adult flies were allowed to lay eggs for one day at 25°, the adults were removed and the vial was shifted to the desired temperature. White prepupae were collected, moved to a new vial and then shifted at the desired time to the desired temperature.

RESULTS

Isolation of a collection of *in* alleles: A collection of 18 new *in* alleles was isolated (Table 1). Ten of our new alleles were isolated after γ-ray mutagenesis, and all of these were examined cytogenetically. Five of these were associated with rearrangements that shared a breakpoint at 77B3-5 (Figure 1). Two of the new alleles and

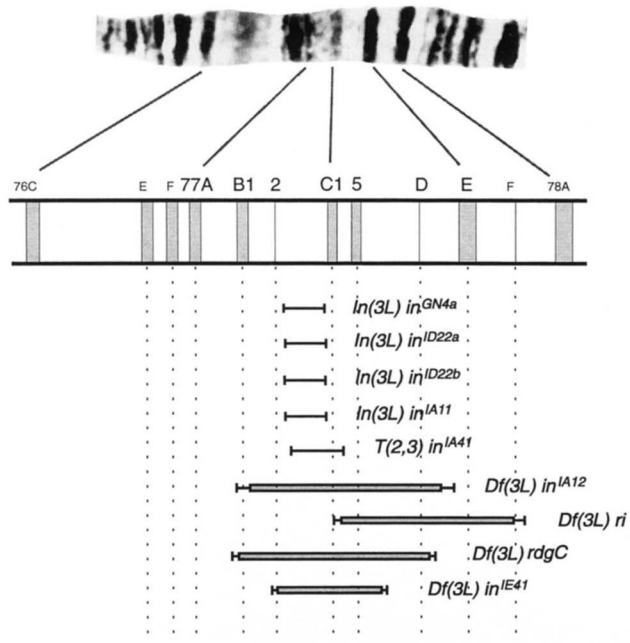

FIGURE 1.—Cytogenetics of *inturned*. Shown is a photograph of the relevant region of 3*L*, an interpretive drawing and our localization of various *in* alleles.

a previously existing deficiency [*Df(3L)rdgC*^{co2}; STEELE and O'TOUSA 1989] that uncovered *in* were associated with cytologically visible deficiencies that removed this region (Figure 1). An additional preexisting deficiency in the region [*Df(3L)ri79c*; JÜRGENS *et al.* 1984] deleted DNA proximal to 77C1, but not 77B3-5. This deficiency removes *ri*, but not *in*. The mapping of the deficiencies was consistent with the assignment of *in* to 77B3-5. This position is close, but slightly distal, to that reported previously for *in* 77C (HANNAH-ALAVA 1971). A casual examination of the thoracic bristle and wing hair phenotypes of the new alleles indicated that all except four of these were similar to the *in*^{1} allele (Table 1). Of these four weak alleles, two (*in*^{GN4a} and *in*^{ID22a}) were associated with heterochromatic breakpoints. The *in*^{GN4a} allele displayed a weak and variable phenotype, and we suggest that it is due to position effect variegation (HENIKOFF 1990; TARTOF *et al.* 1989). The *in*^{ID22a} allele also displayed a weak phenotype, but in this case the phenotype is relatively consistent from fly to fly. Thus, we suggest that this allele is due to a stable position effect. The other two weak alleles were induced by EMS, and are cytologically normal.

***inturned* is not an essential gene:** All of the cytologically normal *in* alleles are viable as a homozygote or as a hemizygote (Table 1). This is also true for two of the rearrangement alleles (*in*^{IA41} and *in*^{IA11}), although the adult flies are very weak and short-lived. Since we isolated a number of cytologically visible deficiencies in our screen, it is clear that the failure to recover any cytologically normal lethal alleles of *in* is not due to an inability to recover null alleles in the screen. Based on these data, we argue that *in* is not an essential gene. The inviability of most of the rearrangements and the weakness of those that are viable suggests that one or more essential genes are located close to, or are interspersed with, *in*.

TABLE 2

The phenotypes of *inturned* alleles

	Fraction of wing showing abnormal polarity[a]		Fraction of multiple hair cells[b]	
Mutation	18°	29°	18°	29°
in^{1}	0.89 (0.02)[c]	0.76 (0.06)	0.79 (0.04)	0.58 (0.13)
in^{HC31}	0.70 (0.03)	0.55 (0.54)	0.73 (0.11)	0.18 (0.07)
in^{IA41}	0.89 (0.01)	0.76 (0.03)	0.78 (0.10)	0.71 (0.08)
in^{II53}	0.29 (0.05)	0.71 (0.04)	0.02 (0.02)	0.58 (0.06)
in^{IH56}	0.90 (0.02)	0.74 (0.03)	0.83 (0.02)	0.68 (0.09)
in^{IH52}	ND[d]	ND	0.75 (0.05)	0.57 (0.09)
in^{HE61}	ND	ND	0.73 (0.05)	0.50 (0.05)
in^{CAH3a}	ND	ND	0.83 (0.09)	0.68 (0.08)
in^{GL4b}	ND	ND	0.78 (0.10)	0.71 (0.06)

All comparisons between the 18° and 29°C phenotypes were significantly different ($P < 0.05$, Mann-Whitney test), except the comparison of *in*^{GL4b} at the two temperatures.

[a] Four wings were scored for each genotype.

[b] Five wings were scored for all genotypes except for *in*^{IA41} and *in*^{CAH3a}, for which we scored 13 and 14 wings, respectively.

[c] The standard deviation is shown in parentheses.

[d] ND, not determined.

Almost all *in* alleles show a cold sensitivity: For a number of strong *in* alleles, we quantified both the fraction of the wing that displayed abnormal hair polarity and the fraction of wing cells forming more than one hair (Table 2). Wings from flies raised at both 18° and 29° were examined. Most of these alleles appeared similar to *in*^{1} on quick observation. For these strong alleles, about 90% of the dorsal wing surface showed grossly abnormal polarity when the flies were raised at 18°, and about 75% of the surface showed abnormal polarity when the flies were raised at 29° (Table 2; Figures 2 and 3). Similarly, about 80% of the cells formed more than 1 hair in wings from flies raised at 18°, and about 70% of the cells formed more than 1 hair in wings from flies raised at 29°. Thus, for both of these phenotypes all of these alleles had a more severe phenotype at 18° than at 29°. We also examined and compared (but did not quantify) the phenotypes of 18° and 29° wings that carried all of the remaining strong *in* alleles. These alleles also appeared to display a more severe phenotype at the low temperature. Since this large set of strong *in* alleles displays the most severe *in* phenotypes that we have detected, we suspect that these represent the null phenotype.

The two "weak" EMS-induced alleles were also analyzed. One of these, *in*^{HC31}, had a more severe phenotype at 18° than at 29° (Table 2; Figures 2, C and D and 4, A and B). Indeed, at 18° its phenotype approached that of the "strong" *in*^{1}-like alleles. It dramatically stood out from the strong alleles by the low frequency (18%) of

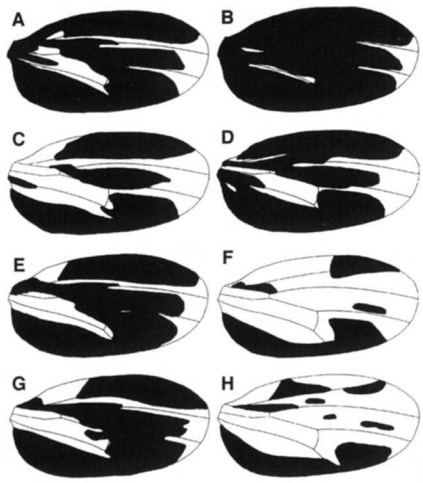

FIGURE 2.—Regions of *in* wings showing abnormal polarity. Shown are drawings where the regions of individual wings that displayed grossly abnormal hair polarity are filled in. The individual wings shown were typical. Drawings similar to these were scanned to determine the fraction of the wing showing abnormal polarity (*e.g.*, Table 2). All wings were from hemizygotes. (A) in^{IH56}/Df, raised at 29°; (B) in^{IH56}/Df, 18°; (C) in^{HC31}/Df, 29°; (D) in^{HC31}/Df, 18°; (E) in^{IH53}/Df, 29°; (F) in^{IH53}/Df, 18°; (G) in^{IH53}/Df, grown at 18° until 67 hr awp then shifted to 29°; (H) in^{IH53}/Df, grown at 18° until 67 hr awp then shifted to 29° (this is the same treatment as G; note the dramatic difference).

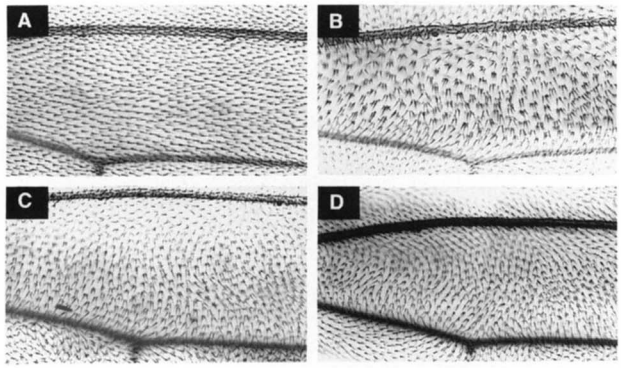

FIGURE 3.—Shown are light micrographs of the dorsal surface of the central region of the C cell of the wing. A small part of the posterior cross vein is shown as a marker. (A) Oregon R (wild type); (B) a region of an in^{CAH3a}/Df wing that displays the large cell phenotype; (C) in^{IH56}/Df, 18°; (D) in^{IH56}/Df, 29°.

multiple hair cells in wings from flies raised at 29° (compared with the approximately 70% for strong alleles) (Table 2). It also had a smaller fraction of the wing with abnormal polarity, but here the difference between in^{HC31} and the strong alleles was not so pronounced (55 *vs.* 75%). The second "weak" *in* allele, in^{II53}, had a very weak phenotype at 18°, with only 2% of the cells forming more than 1 hair, and only 29% of the wing showing abnormal polarity (Table 2; Figures 2, E and F, and 5, A and B). At 29° in^{II53} produced 29 times more multiple hair cells than at 18°. At 29° it had a phenotype that approached that of the strongest *in* alleles.

We attempted to test the hypothesis that the strong alleles were null alleles by comparing the phenotypes of

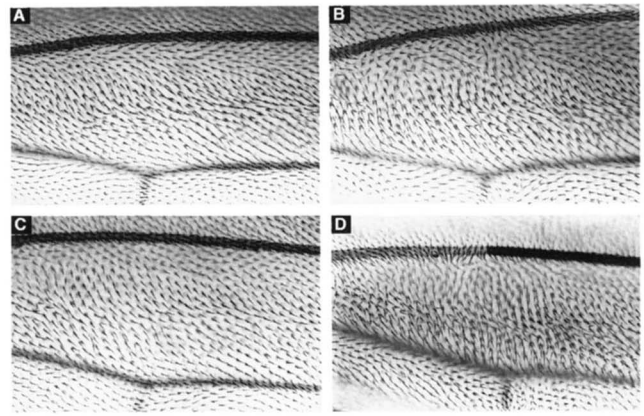

FIGURE 4.—Shown are light micrographs of the dorsal surface of the central region of the C cell of the wing. A small part of the posterior cross vein is shown as a marker. (A) in^{HC31}/Df, 29°; (B) in^{HC31}/Df, 18°; (C) in^{HC31}/Df, animal raised at 29° until 24 hr awp when it was shifted to 18°; (D) in^{HC31}/Df, animal raised at 18° until 72 hr awp when it was shifted to 29°.

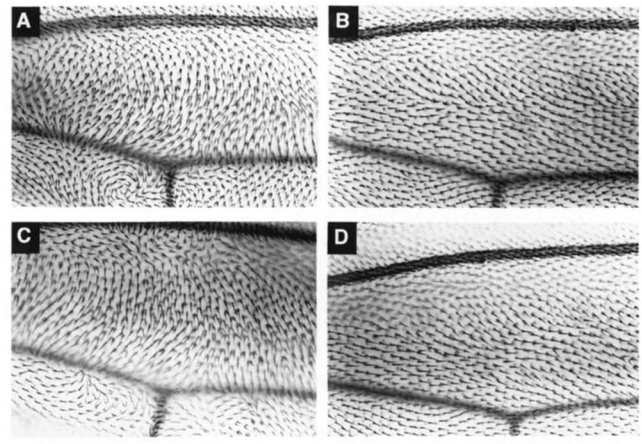

FIGURE 5.—Shown are light micrographs of the dorsal surface of the central region of the C cell of the wing. A small part of the posterior cross vein is shown as a marker. (A) in^{II53}/Df, animal raised at 29°; (B) in^{II53}/Df, animal raised at 18°; (C) in^{II53}/Df, animal raised at 18° until 67 hr awp when it was shifted to 29°; (D) in^{II53}/Df, animal raised at 18° until 67 hr awp when it was shifted to 29°. This is the same condition as in C; note the dramatic difference in the phenotype.

homozygotes and hemizygotes. This was not informative, however, because even for the weak alleles the phenotypes of the homozygotes and hemizygotes was similar. For example, in in^{HC31} homozygous wings from flies reared at 18°, we found that 28% of the cells formed a single hair, compared with the 27% found in in^{HC31} hemizygous wings (Table 2). When similar flies were reared at 29°, we found that 85% of the cells formed a single hair compared with the 82% in hemizygous wings (Table 2). One possible hypothesis to explain the similarity of the hemizygotes and homozygotes is that in the hemizygotes the mutant *in* gene is transcribed at twice the rate of an individual *in* gene in the homozygotes. Many other hypotheses are possible, and it will be necessary to develop molecular probes for the *in* gene products to distinguish among them.

Cold-sensitive period for in^{HC31} ends prior to the start of prehair morphogenesis: We carried out a series of temperature shift experiments to determine when the cold-sensitive period of in^{HC31} was. In preliminary experiments we found that the temperature during larval development did not have any phenotypic effect. For example, when animals were shifted from the restrictive to permissive temperature at white prepupae, a permissive phenotype resulted that was indistinguishable from that seen when animals were reared entirely at the permissive temperature. We concentrated, therefore, on temperature shifts in the early pupal period. In these experiments we only quantified the number of multiple hair cells, because the difference between the two temperatures was much greater for this phenotype than for the fraction of the wing showing abnormal polarity (Table 2). We found that shifting animals to the permissive temperature at or after the first sign of prehair formation [the first sign of prehair formation is seen at 36 hr after white prepupae (awp) at 29° and 72 hr awp at 18°] had no phenotypic effect. For example, shifting animals from the restrictive to the permissive temperature at 72 hr awp did not result in any rescue of the restrictive phenotype (Figures 4D and 6A). There was no unique time prior to prehair initiation when animals needed to be at the permissive temperature to get the permissive phenotype. Rather, incubation at the permissive temperature for a few hours during any period of time prior to prehair initiation provided substantial rescue. For example, one could shift flies from the restrictive to the permissive temperature at 65 hr awp and get good rescue (Figure 6A). In this case the animals were at the permissive temperature only late in the period between white prepupae formation and prehair initiation. Alternatively, shifting animals from the permissive to the restrictive temperature at 9.5 hr awp also resulted in wings where the mutant phenotype was substantially rescued (Figure 6B). In this case the animals were at the permissive temperature only early in the period between white prepupae formation and prehair initiation. We also found that the greater the length of time at the permissive temperature prior to a shift to the restrictive temperature, the greater was the amount of rescue (Figure 6B). In an additional set of experiments animals were given two shifts: for example, a shift from 18° to 29° followed several hours later by a second shift back to 18°. In these experiments, we also found that there was no particular time between white prepupae formation and a few hours prior to prehair initiation when animals needed to be at the permissive temperature for at least partial rescue of the restrictive phenotype (data not shown).

Heat-sensitive period for in^{II53} is very short and a few hours prior to prehair initiation: Our initial temperature shift experiments with in^{II53} showed that the temperature during larval development was unimportant

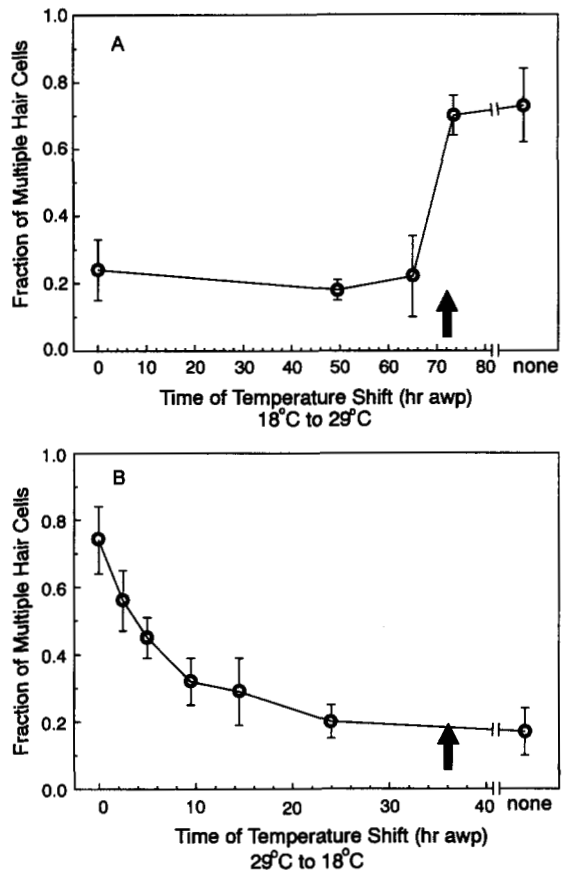

FIGURE 6.—Fraction of multiple hair cells in in^{HC31}/Df wings as a function of the time of temperature shift. A shows shifts from 18° to 29°, and B shows shifts from 29° to 18°. Five wings were scored for all points. Arrows indicate the time of prehair initiation at 18° (A) and 29° (B). Pupal wing development at 29° proceeds at approximately the same rate as is seen at 25°, and at approximately twice the rate seen at 18°.

for the wing phenotype. We next carried out a set of temperature shift experiments in which animals were shifted during the first half of pupal life. In these experiments, we quantified only the fraction of multiple hair cells (Figure 7), but it was clear from simple observation that similar conclusions would be reached from quantifying the fraction of the dorsal wing surface that displayed abnormal polarity (Figure 5). Temperature shifts a couple of hours prior to prehair initiation were too late to have any phenotypic consequences. For example, shifting animals from 29° to 18° at 31.5 hr awp (3.5 hr prior to prehair initiation) provided no rescue of the 29° phenotype (Figure 7B). Shifting animals at earlier times showed that the temperature during much of the early period was unimportant. For example, shifting animals from 18° to 29° at 64 hr awp (8 hr prior to prehair initiation) resulted in a restrictive phenotype that was indistinguishable from that seen in flies raised entirely at 29° (Figure 7A). When animals were shifted from 18° to 29° at 67 hr awp (5 hr prior to prehair formation) or from 29° to 18° at 30 hr awp (6 hr prior to prehair initiation), three types of wings were obtained

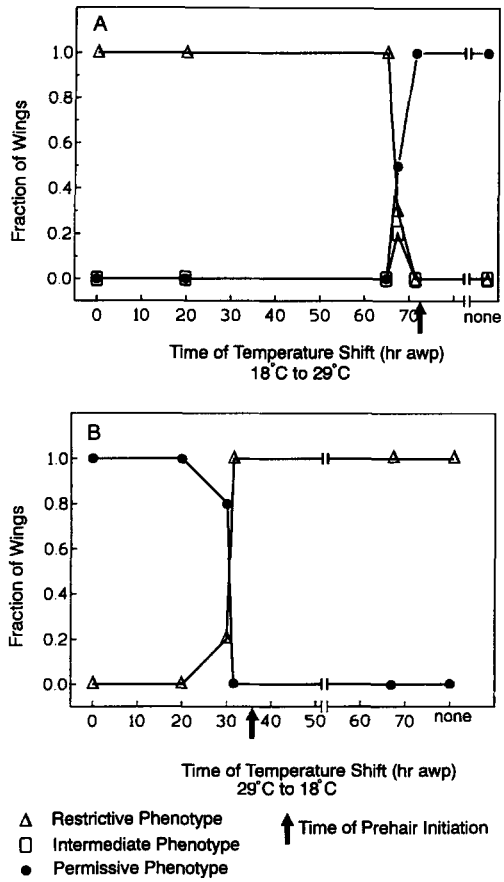

FIGURE 7.—Fraction of in^{II53}/Df wings showing the restrictive, permissive or an intermediate phenotype as a function of the time of temperature shift. Wings where the fraction of multiple hair cells was ≥40% were considered restrictive, 10–39% intermediate, and ≤9% permissive. Arrows indicate the time of prehair initiation at 18° (A) and 29° (B). Unless noted below, five wings were scored for each point. In A, 12 wings were scored for the 71-hr awp time point, and 45 wings for the 67.5 awp hr time point. In B, 10 wings were scored for the 30-hr awp point.

(Figures 5 and 7). Some wings displayed a typical restrictive phenotype (Figure 5C), some displayed a typical permissive phenotype (Figure 5D) and a small number showed an intermediate phenotype. We interpret these data as indicating that the temperature-sensitive period for in^{II53} is very short, and a few hours prior to prehair initiation.

Rare additional phenotype of *in* on the wing: Examining 593 *in* mutant wings, we found 12 wings that displayed an additional phenotype. These wings contained an area with cells that appeared larger and which often appeared to have a stronger than typical *in* phenotype (Figure 3B). The distance between hair clumps was larger, suggesting that the cells that formed the hairs were also larger. One possibility is that the cells that formed these hairs were polyploid. Given the low frequency at which this phenotype was detected, it was not feasible to examine this phenotype in pupal wings. This phenotype was detected in flies that carried four different *in* alleles, including both homozygotes and hemizygotes. Included among the alleles involved were alleles which were isolated in different mutant screens, and which were induced in different genetic backgrounds. This suggests that the phenotype is due to the consequences of the *in* mutations and not to other genetic effects. We have not seen similar patches in the large number of *fz* mutant wings we have examined over the years (*e.g.*, ADLER *et al.* 1987).

DISCUSSION

Is the *inturned* dependent process cold-sensitive? Almost all of our *in* alleles display a stronger phenotype at 18° than at 29°. This could be due to the In protein being inherently cold-sensitive, or it could be due to the *in*-dependent process being cold-sensitive so that the consequences of a lack of *in* activity are more severe at 18° than at 29°. Among the alleles that display the cold sensitivity are alleles that give the strongest *in* phenotype we have been able to obtain and, hence, which we think are likely to be phenotypic null alleles. One of these (in^{IA41}) is associated with an inversion. If one or more of these mutations is a null allele, then it would appear that the process is cold-sensitive. This seems quite likely, but additional molecular data will be required to determine if any of these mutations are indeed null alleles.

In *in* mutant pupal wing cells, prehair formation is not restricted to the distal vertex of the cell (WONG and ADLER 1993). Instead, prehairs form at a variety of locations along the cell periphery. The abnormal polarity of the prehairs appears to be due to their being formed at alternative locations along the cell periphery. The formation of more than one prehair is likely due to prehair initiation no longer being restricted to a small region of the cell (*i.e.*, more than one organizing center can form). Thus, both the abnormal polarity and multiple hair cell phenotypes likely have as a common origin the spread over a larger part of the cell periphery of permissive conditions for prehair initiation. At low temperatures in *in* wings, it seems likely that the size of this permissive region is further increased. The basis for this is unclear, but one attractive (albeit speculative) idea is that it involves the microtubule cytoskeleton. Microtubules are inherently cold-sensitive structures (DUSTIN 1984). In yeast, mutations in the *cin* (chromosome instability) genes have been isolated both by screening for chromosome instability and by super sensitivity to the microtubule-destabilizing drug benomyl (HOYT *et al.* 1990; STEARNS *et al.* 1990). Null mutations in these genes are cold-sensitive and, at the restrictive temperature, mutants appear to have unstable microtubules. There is good precedent in Drosophila for the microtubule cytoskeleton being essential for localizing a signal to a specific location in a cell. The localization of *bicoid* maternal mRNA to the anterior pole of the Drosophila egg requires the function of the microtubule cytoskeleton

(POKRYWKA and STEPHENSON 1991). One of the genes that is essential for the localization of the *bcd* mRNA is the *swallow* gene (BERLETH *et al.* 1988). A second phenotype associated with mutations in this gene is abnormal nuclear divisions in the early embryo. Interestingly, all five *swallow* alleles studied are cold-sensitive for this phenotype (HEGDE and STEPHENSON 1993). Further work will be needed to determine whether the microtubule cytoskeleton is required for restricting prehair formation to the distal vertex of pupal wing cells, whether this is the cause of the cold sensitivity of *in* and whether this system also involves the localization of an mRNA to a particular region of a cell to make that region of the cell unique. The function of the *in* gene could be to regulate the activity of a component or target of such a system. A connection between *in* and the microtubule cytoskeleton might also provide an explanation for the occasional patches of large cells. A disruption of the microtubule cytoskeleton could lead to a failure of mitosis and the formation of a clone of polyploid cells.

One unusual aspect of the cold sensitivity of in^{HC31} is that there is no specific time during the early pupal period when the *in* mutant animal must be at the 29° to relieve the consequences of development at 18°. Perhaps once the cold-sensitive process has taken place (*e.g.*, formation of a certain density of cytoskeleton) it is relatively stable until after prehair initiation.

***inturned* gene functions prior to prehair initiation:** The in^{II53} temperature-sensitive period was very short. Indeed, intermediate phenotypes were quite rare, suggesting that it is more appropriate to consider the temperature-sensitive period to be a temperature-sensitive point. This point is several hours prior to the first sign of prehair morphogenesis (WONG and ADLER 1993). The interpretation of temperature shift experiments is always complicated by the fact that there are a number of possible ways in which a mutation can cause temperature sensitivity. For example, the mutation could result in the protein product of the gene being temperature-sensitive for activity. When this is the case, the temperature-sensitive period corresponds to the essential time of action of the protein. There are, of course, other ways in which a mutation can cause temperature sensitivity. For example, a mutation can result in a protein that becomes unstable at the restrictive temperature. In this case, the temperature-sensitive period would not necessarily correspond to the essential time of action of the protein. The very short temperature-sensitive period of in^{II53} seems most likely to be due to the mutation causing temperature-sensitive activity, but a rigorous answer will require the application of alternative experimental approaches. The observation that the temperature during the entire period of hair morphogenesis is irrelevant for the phenotype of in^{II53} flies is consistent with the previous suggestion that the *in* gene functions to regulate the initiation of prehairs (WONG and ADLER 1993). It is also consistent with the only role for the *in* gene in hair morphogenesis being the regulation of prehair initiation.

***inturned* temperature-sensitive point is at the end of the *frizzled* cold-sensitive period:** A similar set of temperature shift experiments have been done on a cold-sensitive allele of *fz* (ADLER *et al.* 1994). The cold-sensitive period spanned about 24 hr (at 29°) and ended a few hours prior to the first sign of prehair formation (approximately 31 hr at 29° and 67 hr at 18°). Thus, the in^{II53} heat-sensitive point corresponds fairly closely to the end of the *fz* cold-sensitive period. These results are consistent with the suggestion (WONG and ADLER 1993) that *fz* is upstream of *in* in a signaling-signal transduction pathway that controls hair polarity via regulating the subcellular location for prehair initiation. We attempted to do shift experiments on $fz^{cs}in^{II53}$ double mutants to see whether the in^{II53} temperature-sensitive point was after the fz^{cs} cold-sensitive period. We unexpectedly found, however, that the fz^{cs} mutation served as a strong enhancer of in^{II53} so that it no longer showed the marked temperature sensitivity. This and other interactions between *fz* and *in* are under further study in this laboratory.

The time of the in^{II53} temperature-sensitive point is also just prior to the time found by PETERSEN and MITCHELL (1987) for the heat-inducible *multiple wing hair* phenocopy. This is consistent with the suggestion that *in* functions upstream of *mwh* in regulating prehair initiation (WONG and ADLER 1993).

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