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

Paul N. Adler, Jeannette Charlton and Woo Jin Park

Biology Department, Molecular Biology Institute, and Cancer Center, University of Virginia, Charlottesville, Virginia 22903

Manuscript received January 31, 1994
Accepted for publication March 30, 1994

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 (fj)] 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 in1. 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...
partially rescue. One new in allele differed sharply from the others in that it displayed a very weak phenotype at 18°C, but a fairly strong phenotype at 29°C. 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°C. 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 females. The progeny of such crosses were screened for the abnormal bristle polarity phenotype of in (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, y-ray and hybrid dysgenesis mutagenesis was performed as described for F1 mutant hunts for new in 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 %Y(3L)regC. 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 (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 x 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. 1993). We typi- cally 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. 1993). We typically scored five individual wings for a genotype/temperature condition.

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°C, 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 y-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
a previously existing deficiency [Df(3L)rdgC1]; 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] JURGENS 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' allele (Table 1). Of these four weak alleles, two (in'Gv4a and in'D22a) were associated with heterochromatic breakpoints. The in'Gv4a 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'D22a 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'A41 and in'A11), 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.

**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' 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'H驰1, 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' alleles. It dramatically stood out from the strong alleles by the low frequency (18%) of

---

**TABLE 2**

<table>
<thead>
<tr>
<th>Mutation</th>
<th>18°</th>
<th>29°</th>
<th>18°</th>
<th>29°</th>
</tr>
</thead>
<tbody>
<tr>
<td>in'B4</td>
<td>0.89 (0.02)</td>
<td>0.76 (0.06)</td>
<td>0.79 (0.04)</td>
<td>0.58 (0.13)</td>
</tr>
<tr>
<td>in'A41</td>
<td>0.70 (0.03)</td>
<td>0.55 (0.54)</td>
<td>0.75 (0.11)</td>
<td>0.18 (0.07)</td>
</tr>
<tr>
<td>in'A11</td>
<td>0.89 (0.01)</td>
<td>0.76 (0.03)</td>
<td>0.78 (0.10)</td>
<td>0.71 (0.08)</td>
</tr>
<tr>
<td>in'D22a</td>
<td>0.29 (0.05)</td>
<td>0.71 (0.04)</td>
<td>0.02 (0.02)</td>
<td>0.58 (0.06)</td>
</tr>
<tr>
<td>in'D22a</td>
<td>0.90 (0.02)</td>
<td>0.74 (0.03)</td>
<td>0.85 (0.02)</td>
<td>0.68 (0.09)</td>
</tr>
</tbody>
</table>

All comparisons between the 18° and 29°C phenotypes were significantly different (P < 0.05, Mann-Whitney test), except the comparison of in'D22a at the two temperatures.

* Four wings were scored for each genotype.
* Five wings were scored for all genotypes except in'A41 and in'H驰1, for which we scored 13 and 14 wings, respectively.
* The standard deviation is shown in parentheses.
* ND, not determined.
FIGURE 2.—Regions of \textit{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) \textit{in} \textit{wts}/\textit{Df}, raised at 29°; (B) \textit{in} \textit{wts}/\textit{Df}, 18°; (C) \textit{in} \textit{Hc3}/\textit{Df}, 29°; (D) \textit{in} \textit{Hc3}/\textit{Df}, 18°; (E) \textit{in} \textit{wts}/\textit{Df}, 29°; (F) \textit{in} \textit{wts}/\textit{Df}, 18°; (G) \textit{in} \textit{wts}/\textit{Df}, grown at 18° until 67 hr apw then shifted to 29°; (H) \textit{in} \textit{wts}/\textit{Df}, grown at 18° until 67 hr apw 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 \textit{C} cell of the wing. A small part of the posterior cross vein is shown as a marker. (A) Oregon \textit{R} (wild type); (B) a region of an \textit{in} \textit{caH3}/\textit{Df} wing that displays the large cell phenotype; (C) \textit{in} \textit{wts}/\textit{Df}, 18°; (D) \textit{in} \textit{wts}/\textit{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 \textit{in} \textit{Hc3} and the strong alleles was not so pronounced (55 vs. 75%). The second “weak” \textit{in} allele, \textit{in} \textit{wts}, 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° \textit{in} \textit{wts} produced 29 times more multiple hair cells than at 18°. At 29° it had a phenotype that approached that of the strongest \textit{in} alleles.

We attempted to test the hypothesis that the strong alleles were null alleles by comparing the phenotypes of \textit{in} 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 \textit{in} \textit{Hc3} homozygous wings from flies reared at 18°, we found that 28% of the cells formed a single hair, compared with the 27% found in \textit{in} \textit{Hc3} 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 \textit{in} gene is transcribed at twice the rate of an individual \textit{in} gene in the homozygotes. Many other hypotheses are possible, and it will be necessary to develop molecular probes for the \textit{in} gene products to distinguish among them.

FIGURE 4.—Shown are light micrographs of the dorsal surface of the central region of the \textit{C} cell of the wing. A small part of the posterior cross vein is shown as a marker. (A) \textit{in} \textit{Hc3}/\textit{Df}, 29°; (B) \textit{in} \textit{Hc3}/\textit{Df}, 18°; (C) \textit{in} \textit{Hc3}/\textit{Df}, animal raised at 29° until 24 hr apw when it was shifted to 18°; (D) \textit{in} \textit{Hc3}/\textit{Df}, animal raised at 18° until 72 hr apw when it was shifted to 29°.

FIGURE 5.—Shown are light micrographs of the dorsal surface of the central region of the \textit{C} cell of the wing. A small part of the posterior cross vein is shown as a marker. (A) \textit{in} \textit{wts}/\textit{Df}, animal raised at 29°; (B) \textit{in} \textit{wts}/\textit{Df}, animal raised at 18°; (C) \textit{in} \textit{wts}/\textit{Df}, animal raised at 18° until 67 hr apw when it was shifted to 29°; (D) \textit{in} \textit{wts}/\textit{Df}, animal raised at 18° until 67 hr apw when it was shifted to 29°. This is the same condition as in C; note the dramatic difference in the phenotype.
Cold-sensitive period for \( \text{in}^{\text{HC3}} \) 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 \( \text{in}^{\text{HC3}} \) 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 \( \text{in}^{\text{H35}} \) is very short and a few hours prior to prehair initiation: Our initial temperature shift experiments with \( \text{in}^{\text{H35}} \) showed that the temperature during larval development was unimportant 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.
ent 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*141) 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 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 bed 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 polyd ecosystems.

One unusual aspect of the cold sensitivity of in^153 is that there is no specific time during the early pupal period when the in mutant animal must be at the 29°C to relieve the consequences of development at 18°C. 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^153 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^153 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^153 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°C) and ended a few hours prior to the first sign of prehair formation (approximately 31 hr at 29°C and 67 hr at 18°C). Thus, the in^153 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^in^153 double mutants to see whether the in^153 temperature-sensitive point was after the fz^in^153 cold-sensitive period. We unexpectedly found, however, that the fz^in^153 mutation served as a strong enhancer of in^153 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^153 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).

We thank Rani Krasnow and Lily Wong for their helpful comments and suggestions over the years. This work was supported by a grant from the National Institutes of Health (GM37136).

**LITERATURE CITED**


Communicating editor: R. E. Denell