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Antenna to Leg Transformation: Dynamics of Developmental Competence

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ABSTRACT We explore the transformation of antenna to leg in *Drosophila melanogaster*, using ectopically expressed transgenes with heat shock promoters: heat shock *Antennapedia*, heat shock *Ultrabithorax*, and heat shock mouse *Hox A5*. We determined the frequency of transformation of several leg markers in response to *Antennapedia* protein delivered by heat shock at different times and doses. We also studied stage-specific responses to the transgene, heat shock mouse *Hox A5*. Results show that each marker has its own stage and dose-specific pattern of response. The same marker could pass through a period of high-dose inhibition followed by a dose-independent response and then a positive dose-dependent phase. The heat shock-induced transgenes and *spineless aristapedia* transformed the *apterous* enhancer trap antenna disc expression pattern toward the pattern found in leg discs. These results are considered in relation to developmental competence—the ability of developing tissue to respond to internal or external influences. The results suggest that all genes tested interact with the same competence system and that at least two classes of mechanisms are associated with antenna to leg transformation: one comprises global mechanisms that permit transformation over approximately 24 hr; the second class of mechanisms act very locally and are responsible for changes in dose response on the order of 4–8 hr. Dev Genet 19:333–339, 1996.

Key words: *Antennapedia, Ultrabithorax, Sex combs reduced,* competence, homeosis, *apterous, spineless-aristapedia*

INTRODUCTION

The transformation of antenna to leg in *Drosophila* has been used to explore a variety of developmental phenomena. Using transgenes in which the *Antennapedia* (*Antp*) coding sequence is placed under the control of a heat shock promoter, considerable information about the timing of different aspects of antenna to leg transformation has been accumulated [Gibson and Gehring, 1988; Scanga *et al.*, 1995]. Previous studies have revealed that the antenna disc has a wave of transformation sensitivity to *Antennapedia* protein (ANTP). Presumptive distal regions of the antenna transforming into distal leg parts earlier in the sensitive period than presumptive proximal antenna transforms toward proximal leg. This wave of transformation sensitivity holds for both morphological transformation and antenna to leg transformation of antenna disc enhancer trap expression patterns that respond to ANTP [Scanga *et al.*, 1995]. Not only is the sensitivity to ANTP stage dependent, it is also dose dependent. Scanga *et al.* [1995] showed that at 56 hr after hatch, the degree of transformation is modulated by changing heat shock duration of a fly stock with a heat shock-driven *Antennapedia* cDNA (hs*Antp*). The dose of ANTP can be increased by increasing the duration of heat shock at a given temperature or by changing the heat shock temperature. The mutation *spineless-aristapedia* (ss^a) and chemical agents such as borates [Ashburner, 1989a] can transform antennae to leg-like structures, as can heat treating transgenic fly stocks with heat shock promoters fused to *Hox A5* (mouse homolog of fly *Sex combs reduced*, previously designated *Hox 1.3*) [Zhao *et al.*, 1993] or *Ultrabithorax* (*Ubx*) [Mann and Hogness, 1990]. See Lindsley and Zimm [1992] for description of mutants. Because a stimulus promoting antenna to leg transformation can be turned on at any time and to varying degrees during development, the antenna disc provides an excellent system for exploring the nature of developmental competence.

Competence, the ability of developing tissues to respond to internal or external influences, is considered by the embryologist Niewkoop to be the most important problem in developmental biology [Gordon *et al.*, 1994]. Nevertheless, most studies have concentrated on the question: How does a given agent modify development?, rather than: What is the nature of the developmental system responding to the agent? Understanding competence requires that we address this latter

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question. A fundamental aspect of competence is that there exist definable time periods during development when given developing tissues respond to particular agents. This time period is often referred to as a sensitive period. Usually, the tissue is competent to respond to more than one agent. This was amply demonstrated by the variety of "unnatural" inducers that stimulated neural tube formation in vertebrate development [Balinsky, 1970]. As mentioned, antenna–leg transformation in *Drosophila,* the topic of this study, can result from the ectopic expression of at least three proteins in the antenna imaginal disc, the larval precursor of the adult antenna. The antenna–leg transformation illustrates another aspect of competence: it is a multigenic, multicellular phenomenon involving the coordination of both cell differentiation and morphogenesis.

Ultimately we would like to explain the material basis of competence. Before we can do this, it will probably be necessary to first define how many different sorts of processes are involved. We can distinguish phenomena from one another if they occur over different time scales. For example, molecular reactions occur over time scales of parts of seconds while cellular changes may require a time period on the order of hours. Defining the time scales of various responses in a competent system would be a first step in a coherent research program to move from an operational definition of competence to a material one.

In this paper, we study the dose-response dynamics (e.g., changes over time) of the antenna disc exposed to ANTP and other proteins from heat shock promoter-driven transgenes that transform antennae into leg-like structures. To assess the timing of changes in sensitivity to antenna–leg transformation, we vary the dose of ectopic protein in the antenna disc by varying the duration and temperature of heat shock over the course of the sensitive period. Unexpectedly, varied sensitivities for transformation were found both spatially and temporally. The results are consistent with the possibility of at least two different kinds of processes underlying competence.

MATERIALS AND METHODS

Fly Lines

Homozygous hsp70-*Antp* cDNA (hs*Antp*) transgenic line [Zeng *et al.,* 1993] was used in dose-response experiments. Double transgenic lines were constructed between this line and the lacZ enhancer trap line *apterous* (*ap'*), rk568 [Cohen *et al.,* 1992] as described by Scanga *et al.* [1995], creating a *Cy/ap'*, hs*Antp/Sb* line. Homozygous males in this line have low fertility. *ap'* was also bred into lines with heat shock transgenes hs*Ubx* [Mann and Hogness, 1990], hs*Hox A5* [Zhao *et al.,* 1993], and ss^a, all of which have been shown to transform antenna toward leg. The heat shock transgene stocks were maintained as double heterozygotes.

Collection of Timed Larvae and Heat Shock Protocols

Flies were reared on a standard cornmeal medium fortified with minerals and vitamins [Scanga *et al.,* 1995], as well as 10 ml of 10% 4 methylhydroxybenzoate/1,000 ml H_2O, and 100 mg ampicillin/1,000 ml H_2O. For collection of newly hatched larvae, adults were placed in plexiglass cylinders with nylon mesh glued to one end and a 15 × 90-mm petri dish bottom containing 2% agar with yeast paste smeared at its center, on the other end. After a 22- to 24-hr laying period, hatched larvae were removed. Subsequently, 20 hatched larvae were transferred to each glass vial with media. Larvae were timed within not more than ±1–3/4 hr of hatch. Heat shocks were carried out in a temperature-controlled water bath to within ±1°C, the same water bath being used for a given temperature throughout an experiment. To assess transformation of adult structures, adults and pharate adults were stored in 70% ethanol. Antennal structures were separated from the heads and mounted between two coverslips or between a slide and coverslip in Hoyer's medium [Ashburner, 1989b]. Leg markers were scored on the third antennal segment at ×200 with a Zeiss WL microscope.

Statistical Methods

Where one or two durations of heat shock were used (Table 1; see Fig. 4), the percentage of transformations was considered significantly different if they had nonoverlapping 95% confidence intervals, calculated as

$$\% \pm 1.96\sqrt{pq/n}$$

where p is the percentage transformed, q equals 1-p, and n is the number of antennae observed). In the experiments shown in Table 1 and Figure 4, the average N was 54 and 57, respectively.

Where four heat shock durations were used, standard two-way analysis of variance (ANOVA) computer programs were used (SPSS and Statistix) to determined significant treatment effects (Figs. 1, 2). Where treatments had significant effects, Tukey's multiple range test was used to determine which means were significantly different. For the experiment presented in Figure 1, two replicates were analyzed and finding no significant difference between them, the replicate values were averaged. The average size of a treatment class was 46 per replicate.

The experimental results presented in Figure 2 are based on one replicate. Since the data were not normally distributed, the data were transformed using the 1 + log% transform [Steel and Torrie, 1960]. Because different heat shock durations did not alter the percentage transformation, data were pooled over all durations for a given age before graphing. Thus, each point on the graphs is based on more than 155 observations. Graphs were generated with the Cricket graph program, which also fit the linear regression line.

TABLE 1. Percentage Transformation of Leg Markers in Antenna of hs*Antp* Flies After Larval Heat Shocks at 32°C and 37°C

		Heat shock 32°C Transformations					Heat shock 37°C Transformations				
Age	Duration (min)	N	% claw	% T5	% T4	% apical bristle	N	% claw	% T5	% T4	% apical bristle
48	10	59	0	0	0	0	53	47.2	64.2	28	22.6
	60	51	0	1.8	5.8	21.6[a]	52	67.3	69.2	21.2	9.6
52	10	48	0	0	0	0	55	16.4	56.4	29.1	43.6[c]
	60	66	1.5	0	0	27.3[a]	37	29.7	62.2	18.9	13.5
56	10	50	0	0	0	0	88	3.4	29.6	10.2	45.5
	60	48	0	45.8[a]	0	8.3[a]	40	27.5[b]	87.5[b]	38[b]	77.5[b]
60	10	50	0	0	0	0	49	2.0	8.2	4.1	16.3
	60	59	0	0	0	1.7	44	2.3	72.7[b]	25[b]	56.8

[a]Percentage transformation significantly higher than after 10-min heat shock at 32°C based on nonoverlapping 95% confidence intervals.
[b]Percentage transformation significantly higher than that produced by 10-min heat shock at 37°C based on nonoverlapping 95% confidence intervals.
[c]Percentage transformation significantly higher than that produced by 60-min heat shock at 37°C based on nonoverlapping 95% confidence intervals.

Staining Imaginal Discs for β-Galactosidase

Discs were stained as described by Scanga *et al.* [1995]. The Xgal stain was obtained from Biosynth AG. Discs were mounted on slides in Hoyer's solution and photographed using a WL Zeiss microscope and camera with Kodak T-max film (ASA 100).

RESULTS

Dose Response to ANTP

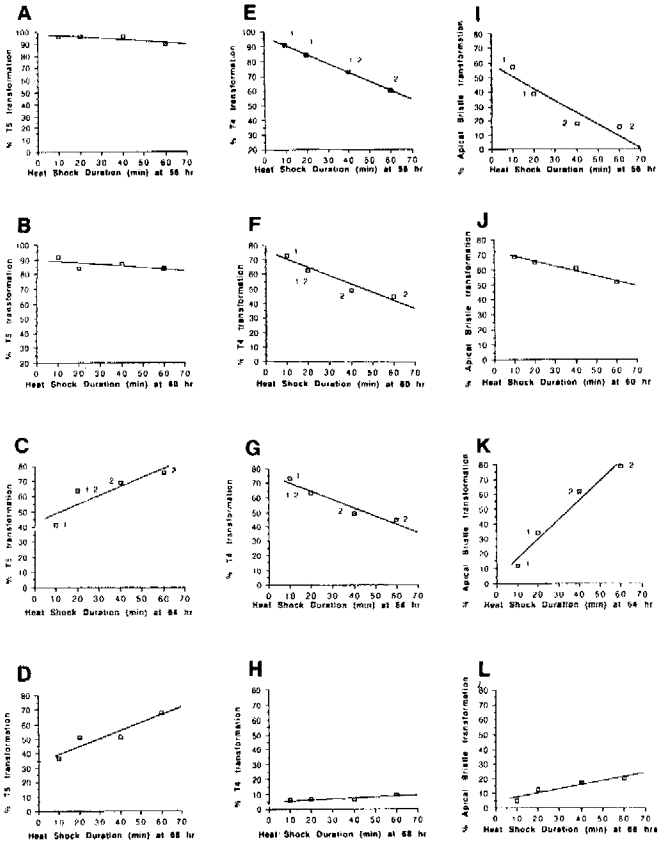
The protein dose from heat shock-driven transgenes can be modulated by changing the duration of heat shock [Manoukian and Krause, 1992] or by changing the heat shock temperature (T. Westwood, personal communication). Figure 1 shows dose-response curves for transformations after 37°C heat shocks of hs*Antp* at 56, 60, 64, and 68 hr after hatch. Data for appearance of tarsal segment 5 (T5), tarsal segment 4 (T4), and apical bristle are presented. Morphological aspects of antenna to leg transformation are illustrated by Scanga *et al.* [1995]. Figure 1A–D shows the dose independence of T5 transformations at 56 and 60 hr, followed by dose dependence at 64 hr. T4 transformations show negative dose dependence until 68 hr, when sensitivity drops to low levels. The frequency of apical bristle transformation shows dramatic changes in pattern from a negative dose response at 56 hr to dose independence at 60 hr and positive dose dependence at 64 hr and low responsiveness thereafter.

It is striking that the competence to make nearby structures varies at a given time and that the time course for these changes in competence can vary independently. For example, while T5 is dose independent at 56 hr and apical bristle and T4 are inversely dose dependent, 8 hr later, both T5 and apical bristle are dose dependent, and T4 is still inversely dose dependent. These results appear to be independent of lethality caused by different heat shock durations, as shown in the tabulations in Figure 1. For example, doubling the length of heat shock from 20 to 40 min has no consistent effect on lethality, although it is associated with statistically significant changes in percent transformation in some aspects of antenna to leg transformations.

In order to interpret inverse dose dependence, it was useful to examine the response to ANTP at a concentration in the neighbourhood of the response threshold. We suspected that a positive dose response would occur at low concentrations; 32°C was chosen as a heat shock temperature because it is at the lower range of temperatures at which the heat shock promoter is activated [Nover, 1991], and a 10-min heat shock was capable of inducing the occasional bracted leg bristle in the third antennal segment of hs*Antp* flies with otherwise normal antenna morphology.

Table 1 compares antenna–leg transformations at 32°C and 37°C at 4-hr intervals between 48 and 60 hr after hatch and for 10- and 60-min heat shocks. With the exception of the previously mentioned leg-like bristles, no transformations toward leg were noted with a 10-min shock at 32°C. With 60-min shocks, apical bristles were produced in frequencies significantly greater than zero at three of the four developmental times tested. The only other significant response was that of T5 at 56 hr. It appears that presumptive apical bristle cells are sensitive to low doses of ANTP in the vicinity of the threshold for response and that at sufficiently high doses, as seen with treatments of 60 min at 37°C, response is indeed inhibited during some time periods (cf. 52- and 56-hr data for 37°C heat shocks).

To determine the dose-response patterns of a putative downstream molecular target of ANTP [Scanga *et al.*, 1995], double transgenic stocks containing hs*Antp* and *ap'*, a β-galactosidase enhancer trap, were heat shocked at 48 hr of larval life for different periods of time at 32°C and 37°C. *ap'* has a different expression



Survival to mature pharate adult after 10- to 60-min. heat shocks

Age at Heat Shock	10 min.	20 min.	40 min.	60 min.
56 hr.	75.0	46.3	66.9	57.5
60 hr.	79.2	62.5	61.7	50.0
64 hr.	71.7	85.8	68.1	63.1
68 hr.	72.5	65.6	63.5	69.4

Fig. 1. Percentage transformation of leg markers after heat shock of hs*Antp* larvae at 37°C for 10, 20, 40, or 60 min were applied to larvae 56, 60, 64, and 68 hr after hatch. **A–D:** Transformation to T5; **E–H:** Transformation to T4; **I–L:** Transformation to apical bristle. Regression lines were fitted by a computer program. Percentage survival is based on the sum of eclosed individuals and those within 1 day of eclosion (pigmented pharate adults).

pattern in leg than in antenna discs [Cohen *et al.*, 1992]. Scanga *et al.* [1995] showed that the antenna disc *ap'* pattern can be transformed to the leg-like pattern in the presence of ANTP. After 37°C heat shocks for 55–60 min of the *ap'* hs*Antp* line, the *ap'* expression pattern was transformed from the knob-like antenna disc pattern to the annulus found in the leg at 48–60 hr after hatch [Scanga *et al.*, 1995] (Fig. 3A,B). At later stages, the sensitive period had passed [Scanga *et al.*, 1995]. After 32°C shocks, the annulus transformation was only found after a 60-min shock, whereas annuli were produced at 37°C after 30-min shocks. It is worth mentioning that some of the larvae heat shocked at 32°C were allowed to develop to adulthood and were then examined for morphological transformation. After 10- or 20-min heat shocks leg-like bristles were found on some AIII segments which seemed morphologically normal. Sensitivity to ANTP is lower for *ap'* pattern transformations than for bristle transformations, since longer heat shock durations were required to produce them.

Antenna to Leg Transformation in Response to hs*Ubx*, hs*Hox A5*, and *ss*^{a}

We wished to obtain evidence that the same underlying competence system in the antenna disc responds to the various genetic perturbations that transform antenna toward leg. This is difficult in the absence of known downstream target genes responsible for the transformation. If the heat shock-driven transgenes *Ubx* and *Hox A5* show the same age and dose-response profile for region-specific transformations as found for hs*Antp*, this would strengthen the assumption that there exists one underlying competence system. We examined adult cuticular structures and the *ap'* expression pattern in third-instar imaginal discs. We also examined the effects of *ss*^{a} on *ap'* expression.

The results of studies with the hs*Hox A5* transgenic stock are presented in Figure 2A–C. Owing to high lethality (>50%), the results must be evaluated with care. The dose-response data were variable, at least in part due to the generally deleterious effects of the *Hox*

A5 protein. In the *ap'*hs*Hox A5* line, transformation competence diminished with increasing age, the claw transformation occurring early with sensitivity dropping off steeply. Because *Hox A5* transforms antenna to a first leg [Zhao *et al.*, 1993], no apical bristles (found only on second legs) were seen. Studies of hs*Ubx*-induced transformations were also hampered by high lethality. Small-scale experiments with hs*Ubx* (data not presented) revealed response patterns similar to those found for hs*Hox A5*.

The effects of hs*Antp*, hs*Ubx*, hs*Hox A5*, and ss^a on the *ap'* expression pattern in the antenna disc is shown in Figure 3C–F. Under appropriate conditions, *ap'* expression pattern transformation occurs in response to the gain of function transgenes and the loss of function mutation ss^a. Figure 4 shows the frequency of *ap'* expression transformation in a hs*Hox A5* background after 10–60 min of heat shock at 37°C. The frequency of partial and complete transformations of the *ap'* pattern drops off markedly after 52 hr for *Hox A5*.

DISCUSSION

Developmental Thresholds: Ranking Leg Marker Transformations

Heat shock-controlled transgenes allow us to explore the interrelationship of gene product quantity, thresholds, and sensitive periods in a way that is unavailable to previous workers. For example, one can inquire whether all aspects of antenna to leg transformation are coordinated in part because they all share the same threshold for sensitivity. Scanga *et al.* [1995] showed that at one developmental stage (56 hr after hatch) the production of claws with 37°C heat shocks occurred after 50-min but not after 40-min heat shocks, suggesting that thresholds for different aspects of transformation might indeed differ. We explored this possibility by reducing the heat shock temperature to 32°C and finding dose responses at several ages; 32°C heat shocks appear to produce responses in the vicinity of transformation thresholds since, in contrast to 37°C treatments, only bracted bristles were found after 10-min shocks. Apical bristles were a consistently produced transformation marker found after 60-min shocks at 32°C, although tarsal segments were also found at some ages. The molecular marker *ap'* showed signs of transformation with 60-min shocks at 48 hr. The claw was found only twice in the 413 adults scored after 32°C heat shocks. We conclude that at low doses of ANTP, the probability of transformation is leg bristles>(tarsal segments, apical bristles, *ap'* pattern)>claw.

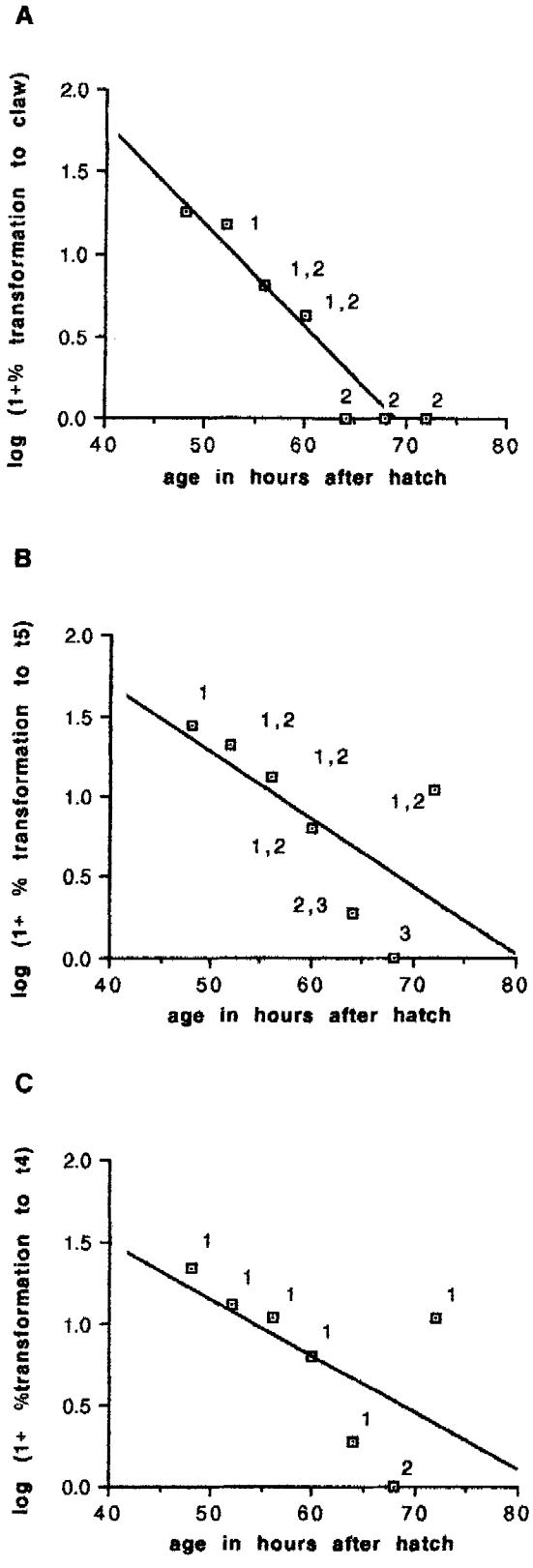


Fig. 2.

Fig. 2. log (1 + % transformation) of leg markers after heat shock of hs*Hox A5* larvae at 37°C, 48, 52, 56, 60, 64, 68, and 72 hr after hatch. Results of heat shock for 10, 20, 40, and 60 min were pooled for each stage. **A:** Transformation to claw. **B:** Transformation to T5. **C:** Transformation to T4. Regression lines were fitted by a computer program.

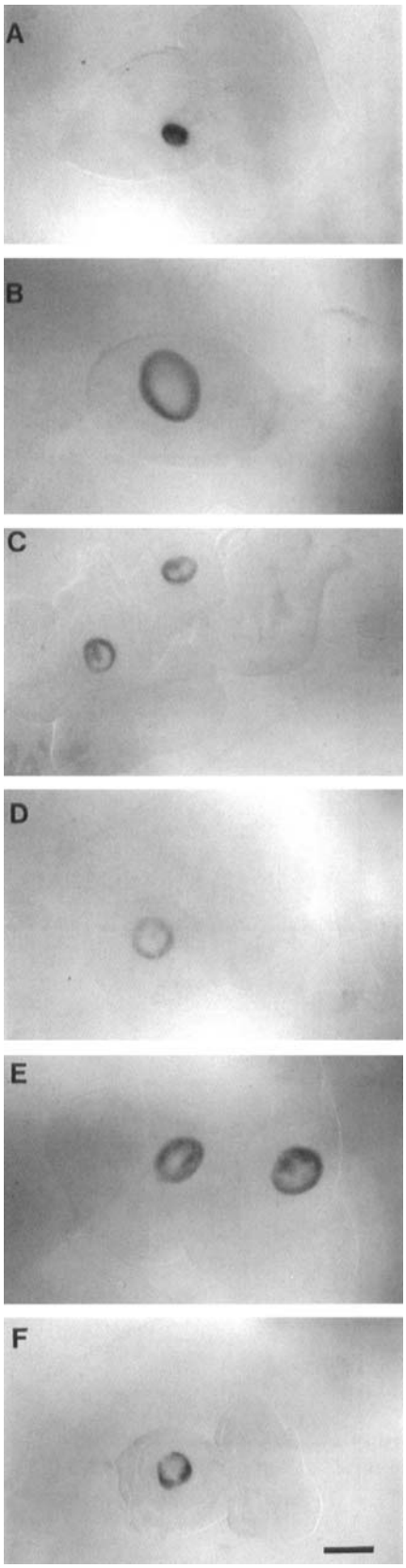


Fig. 3. *ap'* expression pattern in discs from different genetic backgrounds and treatments. **A:** hs*Antp* antenna disc, no heat shock. **B:** hs*Antp* leg disc, no heat shock. **C:** hs*Ubx* antenna disc, heat shocked. **D:** hs*Hox A5* antenna disc, heat shocked. **E:** *ss*^{a} antenna disc. **F:** hs*Antp* antenna disc, heat shocked. The *ap'* expression pattern transformed toward the leg disc pattern in all genotypes under appropriate conditions. Scale bar = 0.1 mm.

Dose-Response Patterns

If dose responses are linear, we can expect three patterns of response: dose-dependent (positive slope), dose-independent (slope of 0), or inversely dose dependent (negative slope). At 32°C, near the transformation concentration threshold, a larger dose produced a response for all markers of transformation except claw. At 37°C, ANTP levels were evidently above the threshold for most markers of transformation, and all three types of dose response were found. Initially the T5 transformation was dose independent, becoming dose dependent toward the end of the sensitive period. By contrast, after initial dose independence at a low frequency of transformation, apical bristle sensitivity went from being inhibited by high doses of ANTP to being dose independent, and finally to dose dependence. Thus, even at a given stage, different populations of cells within the disc will have very different sensitivities to what is assumed to be the same dose of ANTP.

Changes in patterns of dose response were similar from one experiment to the next for T5 and apical bristle transformations. By contrast, T4 was more variable, sometimes behaving more like T5 and sometimes showing inverse dose dependence. The variable T4 transformation dose response is interesting because of its implications for the relationship of transformation competence of serially related structures (e.g., T5–T1), but for our present purposes it is a less reliable marker of transformation than T5 and the apical bristle.

The duration of a dose response mode also varies with the marker observed. For example, for 37°C shocks T5 remained dose independent for at least 8 hr (Fig. 1A,B), whereas apical bristle transformation showed this response at only one time point (Fig. 1J). We do not, at present, know what accounts for these differences. Furthermore, until more is known about the downstream targets of ANTP (and other homeotic genes), we will not know whether the components interacting with ANTP are similar in each presumptive cell type. We can, however, examine transgene expression patterns that are modified by homeotic gene products, to see whether they would provide suitable markers for exploring the molecular basis of competence.

Competence for *ap'* Expression Pattern Transformation

From previous work [Scanga *et al.*, 1995], it is known that competence to transform the *ap'* pattern from an antenna to a leg-like pattern decreases with age, mim-

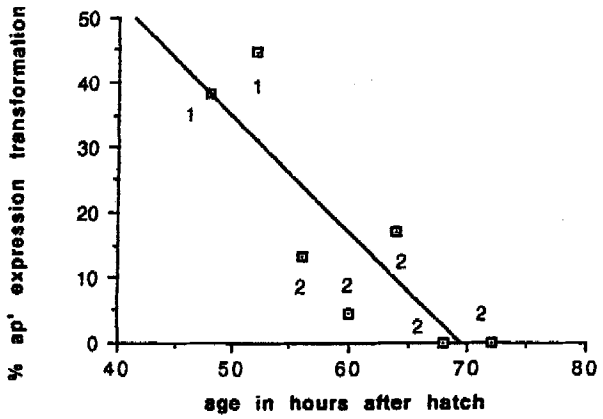


Fig. 4. Percentage transformation of *ap'* disc expression pattern towards a leg-like pattern in *ap'*, hs*Hox A5* larvae after 37°C heat shocks at 48, 52, 56, 60, 64, 68, and 72 hr after heat shock. (Results were pooled for all heat shock durations, which varied from 10 to 60 min.) The regression line was fitted by a computer program.

icking the distal to proximal transformation wave described by others [Gibson and Gehring, 1988]. The *ap'* disc-staining expression pattern transformation also responds in a dose-dependent manner to ANTP. At 48 hr, 37°C shocks elicited transformation after 30-min durations, but 10- or 20-min shocks proved insufficient.

Our present results also show that *ap'* expression patterns transform from antenna to leg-like patterns not only in response to ANTP, but also following heat shocks with hs*Ubx* and hs*Hox A5* and in combination with ss^a. Since *ap'* has little to do with antenna or leg morphogenesis (*ap'* homozygotes lack wings and halteres but have apparently normal antennae and legs), the fact that *ap'* expression patterns are transformed by several transforming agents suggests that there may be some common features shared by targets responding to leg induction. It is striking, for example, that *ap'* pattern transformation responds not only to the heat shock-driven gain of function homeotic genes, but also to a loss of function mutation such as ss^a, which does not require downstream ANTP for antenna to leg transformation [Burgess and Duncan, 1990]. Thus, whether or not *ap'* functions in antenna to leg transformation, the *ap'* transgene pattern transformation in response to ANTP is spatially, temporally, and dose dependent in the way that morphological transformations are and may be used as a downstream target of ANTP in molecular studies.

We have explored aspects of competence for antenna to leg transformation. We have shown unexpectedly complex response dynamics to ANTP. We have also demonstrated commonalities in the response of the antenna disc to several antenna to leg transforming agents. The distal to proximal transformation sensitivity was found to be similar for all three heat shock-promoted transgenes. Furthermore, the transgenes and ss^a all transformed the *ap'* enhancer trap expression pattern in the antenna disc to one usually found in leg discs, suggesting that, for the most part, the same responsive system is activated in each genotype. Thus, enhancer traps like *ap'* should prove useful in testing hypotheses concerning the molecular basis of competence.

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REFERENCES

Ashburner M (1989a): "*Drosophila*, A Laboratory Manual." Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, p 167.

Ashburner M (1989b): "*Drosophila*, A Laboratory Handbook." Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, p 1068.

Balinsky BI (1970): "An Introduction to Embryology." Philadelphia: WB Saunders.

Burgess EA, Duncan I (1990): Direct control of antennal identity by the *spineless–aristapedia* gene of *Drosophila*. Mol Gen Genet 221: 347–357.

Cohen B, McGuffin ME, Pfeifle C, Segal D, Cohen SM (1992): *apterous*, a gene required for imaginal disc development in *Drosophila* encodes a member of the LIM family of developmental regulatory proteins. Genes Dev 6:715–729.

Gibson G, Gehring WJ (1988): Head and thoracic transformations caused by ectopic expression of *Antennapedia* during *Drosophila* development. Development 102:657–675.

Gordon R, Bjorlund NK, Nieukoop PD (1994): Appendix: Dialogue on embryonic induction and differentiation waves. Int Rev Cytol 150: 373–420.

Lindsley DL, Zimm GG (1992): "The genome of *Drosophila melanogaster*." New York: Academic Press.

Mann RS, Hogness DS (1990): Functional dissection of *Ultrabithorax* proteins in *D. melanogaster*. Cell 60:597–610.

Manoukian AS, Krause HM (1992): Concentration-dependent activities of the *even-skipped* protein in *Drosophila* embryos. Genes Dev 6:1740–1751.

Nover L (1991): "Heat Shock Response." Boca Raton, FL: CRC Press, p 7.

Scanga S, Manoukian A, Larsen E (1995): Time- and concentration-dependent response of the *Drosophila* antenna imaginal disc to *Antennapedia*. Dev Biol 169:673–682.

Steel RGD, Torrie JH (1960) "Principles and Procedures of Statistics." New York: McGraw-Hill, p 157.

Zeng W, Andrew DJ, Mathies LD, Horner MA, Scott MP (1993): Ectopic expression and function of the *Antp* and *Scr* homeotic genes: The N-terminus of the homeodomain is critical to functional specificity. Development 118:339–352.

Zhao JJ, Lazzarini RA, Pick L (1993): The mouse *Hox-1.3* gene is functionally equivalent to the *Drosophila Sex combs reduced gene*. Genes Dev 7:343–354.