washing the cells twice measured by ^{3}H-leucine incorporation[2]. Protein synthesis of PBMC was effectively blocked in the presence of PM even at a low dose of 2×10^{-6} M, or by pretreatment of PBMC with emetine HCl (EM, Reanal, Hungary) for 45 min at 37 °C. After EM pretreatment PBMC were washed twice before addition to HEp-2 targets. At the doses applied neither of the drugs affected the viability of effector (trypan blue exclusion) or target cells (unchanged adherence).

Statistics. Data are given as a mean ± SEM of n experiments. Statistical were performed with Student's t-test.

Results and discussion. Blocking of DNA synthesis by MC pretreatment of PBMC had no major effect on either NCMC or LDCC to HEp-2 cells (table 1). Similar results were obtained using ^{51}Cr-labeled K 562 targets[2,9]. In parallel experiments, MC pretreatment completely abrogated Con A-induced lymphocyte proliferation (table 2).

Inhibition of NK activity against K 562 target cells was demonstrated by pretreatment of effector cells with actinomycin D[2]. The relationship of LDCC to RNA synthesis has not yet been investigated. Blocking of RNA synthesis by AD pretreatment of PBMC dose-dependently reduced both LDCC activity and Con A-induced blastogenesis. LDCC (table 1) and lymphocyte proliferation (table 2) were significantly inhibited by AD at a concentration of 2.5 µl/ml (2×10^{-9} M). AD had no major influence on NCMC against HEp-2 cells (table 1). This suggests that contrary to NCMC, LDCC against HEp-2 targets requires the synthesis of new RNA molecules, similar to the Con A-induced proliferation and the NK activity[2] of lymphocytes.

Recently, in the presence of a reversible protein synthesis blocker, puromycin (PM), NK activity against K 562 targets was inhibited but could be easily reversed by washing out the drug, while emetine HCl (EM) irreversible blocked both protein synthesis and NK activity against K 562 targets[2]. Sawada and Osawa have earlier demonstrated inhibition by PM of LDCC in the mouse[15]. Pretreatment of PBMC by PM, even at the high concentration of 10^{-4} M, failed to influence NCMC and LDCC activities, as well as Con A-induced blastogenesis (data not shown). In the presence of PM, even at a low dose of 2×10^{-6} M, a strong stimulation of NCMC ($p < 0.01$), and inhibition of LDCC ($p < 0.05$) occurred (table 1). PM also abrogated Con A-induced blastogenesis (table 2). Though PM in the dose applied effectively blocked protein synthesis, a direct effect of the drug on NCMC and LDCC could not be entirely ruled out. Thus, the relationship of NCMC and LDCC with protein synthesis was also studied using EM, an irreversible protein synthesis blocker[2]. As is shown in table 1, EM dose-dependently enhanced NCMC, and reduced LDCC, similarly to the changes observed in the presence of PM. In parallel experiments Con A-induced blastogenesis of PBMC was also blocked by EM pretreatment (table 2).

The present results confirm earlier observations that both NCMC and LDCC independent of DNA synthesis[2,9]. Furthermore, these data reveal a different relationship between RNA and protein synthesis as well as NCMC and LDCC, respectively. Likewise, the Con A-induced proliferation of lymphocyte LDCC activity also requires de novo RNA and protein synthesis, while NCMS against HEp-2 target cells depends on preformed structures. Very recently, cytotoxic macrophages were shown to perform down regulation of RNA labeling[18], and to have a requirement for protein synthesis upon stimulation by alpha- and beta-interferon but not by gamma-interferon[3]. In contrast to the NCMC against HEp-2 carcinoma cells NK activity towards K 562 leukemia target cells was inhibited by both RNA and protein synthesis blockers[2]. This can be associated with previous data suggesting the heterogeneity of cytotoxic mechanisms against carcinoma/sarcoma/solid tumor/ as well as leukemia/lymphoma target cells in the mouse[7,16]. Augmentation of NCMC by blocking of protein synthesis could be related to the blocking of the synthesis of an inhibitory protein for NCMC by PM and EM, or to effects of PM and EM on suppressor cells for NCMC[17]. This question is currently being investigated in our laboratory.

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Temperature-dependent responses to a developmental gradient in the *Drosophila* wing[1]

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Summary. The mutant hairy *(h)* increases the number of sensillae on the *Drosophila* wing. This allows us to quantify a gradient that determines the type of sense organ that forms along the third long vein. Temperature significantly shifts the positional responses to this underlying gradient.

Key words. Positional information; temperature effects; gradients; campaniform sensillae; wing veins; *Drosophila melanogaster.*

Gradients appear to play a central role in determining the placement of structures in a developmental field[2]. *Drosophila melanogaster,* for example, typically has three domed campaniform sensillae on the third wing vein (L3) distal to the crossvein. Although individuals may differ in the relative placement of these sensillae, their average positions are extremely constant.

Sensillae in wild type flies respond homeostatically to alterations in wing size associated with sex differences or culture temperature[3].

One can detect the gradient underlying this system by measuring the expression of mutants that influence the development of wing sense organs. The mutant hairy (*h*, 3–26.5) increases the number of sense organs on the L3 vein[4,5]. These can be classified into three morphologically different types. Campaniform sensillae predominate on the proximal section of the vein, while bristles are most common nearer the tip. Between these are small transitional sensillae that appear bristle-like and are variable in size.

The distribution of each type can be quantified by first dividing the L3 vein into 20 equal sections to compensate for variation in wing length. Then the position of each sense organ is measured using a microscope eyepiece graticule and is assigned to the appropriate 5% unit of vein length. In the following analysis, positional data will be expressed in terms of these scale values, which increase from 1 at the crossvein to 20 at the distal tip.

The numbers and positions of sense organs were measured in 50 male wings raised at 18 ± 0.5, 25 ± 0.5, and 29 ± 0.5 °C (table). Although there is a slight reduction in the total number of sense organs at low temperature, there is a dramatic change in the proportions of each type. The number of campaniform sensillae almost doubles when culture temperature is reduced from 29 to 18 °C. In contrast, the number of bristles is more than halved. These shifts are approximately linear when plotted against temperature.

The numerical responses to temperature are paralleled in the distributional patterns. The sense organs falling into each of the 5% sections of the L3 vein are summarized in the figure for 18 °C and 29 °C wings. As the number of bristles increases with higher temperature, their frequency in the proximal part of the wing also increases. The transitional organs are intermediate products of the homology between bristles and campaniform sensillae. They also increase in number and become more frequent nearer the base of the wing as temperature increases (for example, the mean position at 18 °C is 10.84 ± 0.38 compared to 7.49 ± 0.23 at 29 °C). Median locations are given in table.

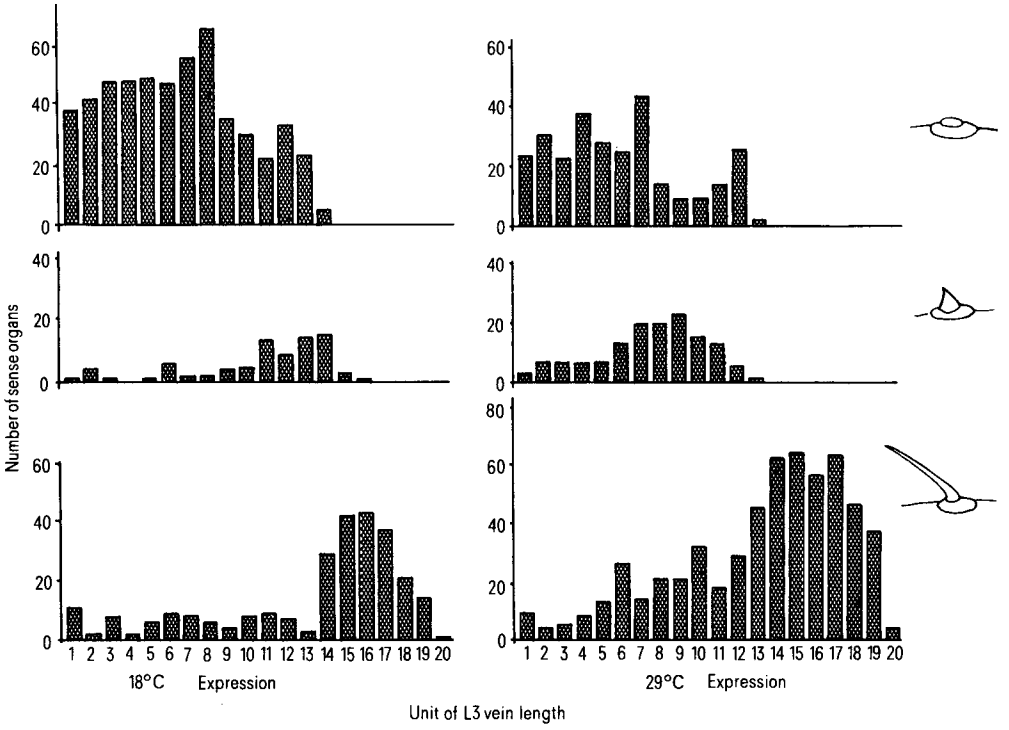
Clearly, while temperature has only a minor effect upon the total number of sense organs, it has a major influence on which of these homologous structures will develop. Furthermore, the shift in frequency follows a simple, predictable pattern. One way to interpret this pattern is to hypothesize a proximal-distal gradient that governs the developmental response of cells along this wing axis. These data support the idea that such a gradient, or at least the response to it, is temperature sensitive.

Other recent studies[3,6] suggest that this gradient is also readily modifiable both by major genes and by polygenic factors. The interaction among these components makes this system a very attractive one for future study of the control and coordination of patterns in development.

Number and placement of campaniform sensillae, intermediate organs and bristles on male *hairy* wings of *Drosophila melanogaster* raised at three temperatures

	Culture temperature		
	18 °C	25 °C	29 °C
Number of sense organs*			
Campaniform sensillae	10.84 ± 0.28	8.64 ± 0.29	5.74 ± 0.18
Transitional structures	1.70 ± 0.16	2.48 ± 0.20	2.80 ± 0.18
Bristles	5.40 ± 0.31	8.94 ± 0.29	11.54 ± 0.31
Total	17.94 ± 0.40	20.06 ± 0.33	20.08 ± 0.32
Relative position**			
Campaniform sensillae	6.48	5.97	5.48
Transitional structures	11.72	9.63	7.85
Bristles	15.05	15.07	14.20

* $\bar{x}$ ± SE; n = 50. ** Median division (5% unit).



Distribution of campaniform sensillae, transitional sense organs, and bristles along the L3 vein of *D. melanogaster* raised at 18 °C and 29 °C.

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