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11. We thank J. M. Feliksik for excellent technical assistance, S. A. Milton for manuscript preparation, and R. Zelka for illustration. Supported in part by grant ESO2397 from the National Institutes of Health and a grant from the Veterans Administration.

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Light-Induced Modification of *Drosophila* Retinal Polypeptides in vivo

Abstract. *The effect of light on the polypeptide map profile of the* Drosophila *eye preparation was examined by two-dimensional polyacrylamide gel electrophoresis. The results show (i) that illuminating the living fly reversibly changes the isoelectric points of three classes of polypeptides specific for the photoreceptor layer and (ii) that the* norpA *mutation, which prevents the generation of the receptor potential, blocks the modifications.*

Posttranslational modification of proteins has been suggested to play a role in regulating various biological systems (*1*). In visual receptor cells, light-dependent posttranslational modifications of proteins that have been reported include light-dependent phosphorylation of rhodopsin (*2*) and other unidentified receptor proteins (*3*) and methylation of rod outer segment proteins (*4*). However, little or no evidence exists to date to suggest that any of these modifications are involved in the visual process.

We report here three classes of retina-specific polypeptides that undergo light-dependent modification in *Drosophila*. The availability of the *norpA* mutation (*5*), which blocks the receptor potential, allowed us to investigate the linkage between these protein modifications and phototransduction.

Eye proteins were separated by two-dimensional gel electrophoresis on the compound-eye or on a photoreceptor-layer preparation (*6*) of wild-type flies of the Oregon-R strain or *norpA* mutant (*5*) flies, according to the Miyazaki *et al.* (*7*) modification of O'Farrell's (*8*) original procedure. The effect of light on the polypeptides was determined by comparing the gels obtained from flies that had been light-adapted for 24 hours, or exposed to a brief light stimulus, with those obtained from flies that had been dark-adapted for 24 hours. White light of moderate intensity (160 $\mu W\ cm^{-2}$) was used throughout our experiments. Flies were prepared for electrophoresis (*9*) by quickly freezing the living flies (less than 5 seconds) in liquid nitrogen after the dark or light treatment, dehydrating them in an acetone and dry ice mixture, and microdissecting out the compound eye or their constituents (*9*).

Portions of the gels obtained from dark-adapted (Fig. 1a) and light-adapted (Fig. 1b) wild-type flies, respectively, were compared; the gels show that three groups of polypeptide spots shift their isoelectric points toward the acidic direction on light adaptation (*10*). These polypeptides are designated 80 K, 49 K, and 39 K spots (80 K indicates 80,000), according to their apparent molecular weights. The changes in the 80 K, 49 K, and 39 K polypeptides brought about by light-adapting the flies are shown in Fig. 1c. These polypeptide changes were observed consistently in more than 80 different samples (*11*). Comparing results obtained from the microdissected photoreceptor-layer preparation (*6*) with those obtained from other portions of the head showed that the 80 K, 49 K, and 39 K spots originated exclusively from the photoreceptor layer (data not shown).

Since the above changes in living flies were observed with a light of moderate intensity, they represent molecular changes that take place in the living eye under physiological conditions. The light-induced changes of the polypeptides occur only in the isoelectric focusing dimension and always consist of shifts toward the anode (that is, acidic direction, Fig. 1b). Although the chemical nature of these changes has not yet been investigated, they could involve phosphorylation of the polypeptides.

In experiments on the approximate time requirements of the light-induced changes and their reverse reactions (Fig. 2, a to d), flies that had been dark-adapted for 12 hours were divided into four groups, and each group was exposed to a different illumination condition. All three classes of polypeptide spots shift to the light state within 5 minutes of illumination (Fig. 2b). In fact, a 10-second illumination was sufficient to induce the shift of both the 80 K and 49 K spots, although not the 39 K spots (not shown). All three classes of polypeptides return to the dark state after the cessation of light stimulus. The time courses of the reverse reactions are slower than those of the forward reactions and also vary among the three classes of polypeptides. Thus, the modification of the 80 K polypeptide (or polypeptides) is reversible within 5 minutes in the dark (Fig. 2c), whereas that of the 49 K polypeptides reverses partially in 5 minutes, and the reversal continues for up to at least 1 hour (Fig. 2d). The 39 K spots do not return to the dark state even after 1 hour of dark adaptation (Fig. 2d); ultimately they do reverse to the dark state (Fig. 2a, 12-hour dark adaptation).

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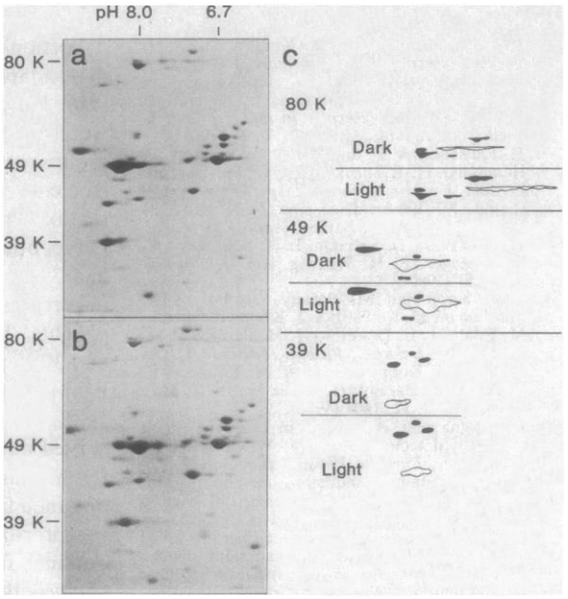

Fig. 1. Light-induced changes of the 80 K, 49 K, and 39 K polypeptides detected by two-dimensional polyacrylamide gel electrophoresis. Only a portion of the gel is shown. The sample consisted of 140 eyes (or 100 eyes in Fig. 2) prepared from flies that had been light- (Fig. 1b) or dark-adapted (Fig. 1a). The samples were homogenized in 70 μl of a solution consisting of 8.5*M* urea, 2 percent Triton X-100, 2 percent Bio-Lyte 3/10 (Bio-Rad), and 5 percent β-mercaptoethanol. The first dimension is isoelectric focusing, run from right (acidic) to left (basic) on 4 percent polyacrylamide with 2 percent Bio-Lyte 3/10. The second dimension is 10 percent polyacrylamide sodium dodecyl sulfate (SDS) gel electrophoresis, from top to bottom (*16*). Gels were stained with Coomassie brilliant blue (*17*). (a) Compound-eye sample dissected from the flies that had been dark-adapted for 24 hours. (b) Compound-eye sample dissected from the flies that had been light-adapted for 24 hours. (c) Schematic representation of the 80 K, 49 K, and 39 K polypeptide spots showing the light-induced changes. Open traces: the 80 K, 49 K, and 39 K polypeptides that undergo light-induced modifications. Filled spots: other spots located close to the 80 K, 49 K, and 39 K spots shown here to serve as internal markers.

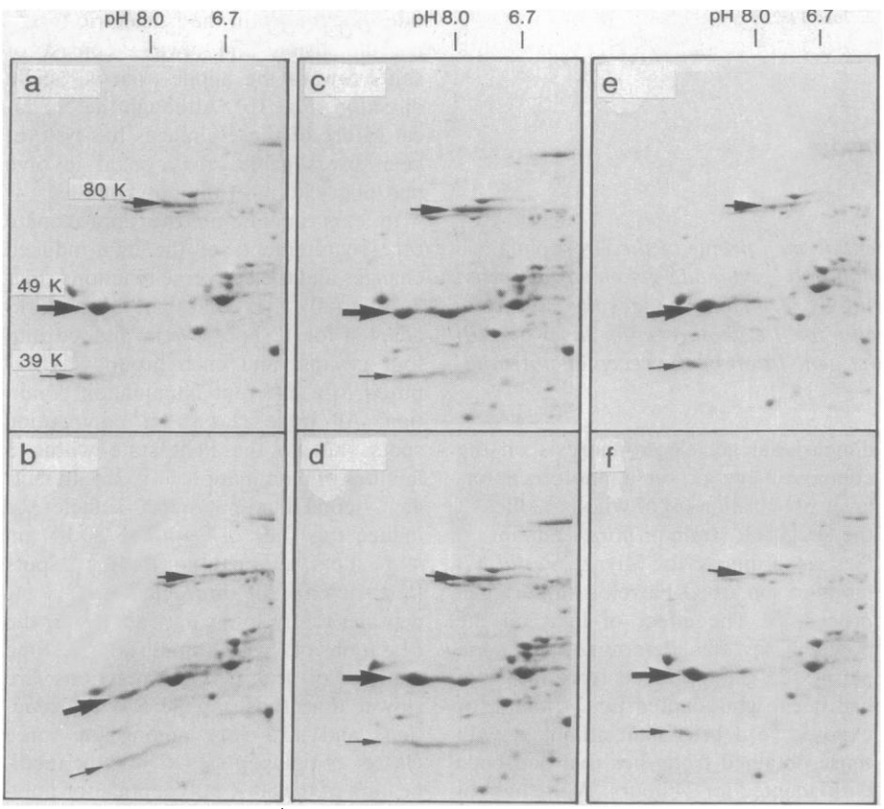

Fig. 2. (a to d) Reversibility of the 80 K and 49 K polypeptide modifications in the dark. (a) Wild-type flies dark-adapted for 12 hours. (b) Wild-type flies exposed to light for 5 minutes after 12 hours of dark adaptation. (c and d) Wild-type flies that had been dark-adapted for 12 hours and then exposed to light for 5 minutes were dark-adapted again for 5 minutes (c) and 1 hour (d), respectively. (e and f) Effect of the $norpA^{P24}$ mutation on polypeptide modifications. (e) *norpA* flies dark-adapted for 12 hours. (f) *norpA* mutant flies that had been dark-adapted for 12 hours were exposed to light for 5 minutes. The three classes of light-modifiable polypeptides are indicated by arrows of different sizes (49 K, large; 80 K, medium; and 39 K, small). The distortion in the SDS-PAGE profile in Fig. 2, b and e, is due to a bias current that was turned on accidentally.

The relatively fast time courses and reversibility of the modification reactions suggest that these changes are due to light-induced posttranslational modification of polypeptides rather than to de novo synthesis of the polypeptides. Moreover, the photoreceptor-layer specificity of the 80 K, 49 K, and 39 K polypeptides, the light-inducible properties of the modification of these polypeptides, the relatively rapid time courses of the modification reactions, and their reversibility in the dark suggest that these polypeptides and the light-induced modification that they undergo are part of the molecular processes of *Drosophila* vision. We have tested this hypothesis with the *norpA* mutant (*no* receptor *p*otential *A*; chromosome map position, 1-6.6 ± 0.7) (*5*, *12*). Previous studies (*12*) have indicated that the *norpA* mutation affects an intermediate step of phototransduction so that the receptor potential is blocked even though the visual pigment undergoes normal phototransitions. In that a reversibly temperature-sensitive *norpA* allele has been isolated, it is likely that the *norpA* gene codes for a polypeptide essential in the intermediate step of phototransduction (*12*). Two mutant alleles of the *norpA* locus have been used in this study: $norpA^{P24}$ and $norpA^{EE5}$. The receptor potential is completely blocked in $norpA^{P24}$, while a very small, degraded receptor potential is present in $norpA^{EE5}$. The light-dependent modifications of the 80 K, 49 K, and 39 K polypeptides were found blocked in both $norpA^{P24}$ (Fig. 2, e and f) and $norpA^{EE5}$ (not shown).

However, since the receptor potential is either small or absent in these *norpA* mutants, depolarization of the photoreceptor membrane could be responsible for the light-induced modifications of the polypeptides. To exclude this possibility, we examined CO_2-treated flies for the polypeptide modifications (*13*) because CO_2 treatment depolarizes the receptor cells in many invertebrates (*14*), including the *Drosophila* photoreceptors (*15*). We found that (i) CO_2-induced depolarization of the receptor membrane does not induce modifications of the polypeptides and (ii) light can induce the modifications regardless of whether or not the photoreceptor membrane is depolarized by CO_2 treatment (data not shown). These results exclude depolarization of the photoreceptor membrane as a causal agent for the light-induced modifications of polypeptides. Inasmuch as the *norpA* mutation blocks an intermediate step of phototransduction (*5*, *11*), our results suggest that the light-induced modifications of these polypeptides depend on

the integrity of the phototransduction process. The relation between the two processes, however, remains to be elucidated. Our results with mutants, nevertheless, support the idea that the light-dependent modifications of polypeptides reflect molecular events occurring in the visual processes of *Drosophila*.

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10. Under these conditions all the proteins are presumably in a denatured state. For this reason we refer to the spots as "polypeptides" rather than "proteins." It may be noted that all three classes of light-modifiable polypeptides show varying degrees of charge heterogeneity in both light and dark states (Fig. 1, a and b). It is not clear whether the multiple spots in each cluster are due to heterogeneous polypeptides that cannot be separated by our experimental procedures or to multiple modification events occurring on a single polypeptide.
11. If two gels—one containing a dark-adapted sample and the other a light-treated sample (Fig. 1, a and b)—are treated identically and processed at the same time, they produce polypeptide patterns that are superimposable upon each other except for the 80 K, 49 K, and 39 K spots. However, because of a slight difference in photographic reproduction and in staining and destaining, some of the minor spots show up more clearly in Fig. 1b than in Fig. 1a. In Fig. 2 the gel patterns are slightly streaky along the first dimension, but all spots can be unequivocally assigned to the corresponding ones in Fig. 1.
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Leukotriene D_4: A Potent Coronary Artery Vasoconstrictor Associated with Impaired Ventricular Contraction

Abstract. *Leukotriene D_4 (2×10^{-9} mole), injected into the left circumflex coronary artery of anesthetized sheep, produced profound coronary vasoconstriction and impaired regional ventricular wall motion. This cardiac effect was neither inhibited by prior treatment of the sheep with a cyclooxygenase inhibitor nor associated with thromboxane B_2 release into the coronary sinus. Intravenous FPL 55712 completely abolished the coronary vasoconstriction of leukotriene D_4, but a significant reduction of regional wall shortening persisted.*

Hypotensive shock and cardiac failure are clinical manifestations of systemic anaphylaxis (*1*). Electrocardiographic changes characteristic of coronary artery spasm have been reported during human anaphylaxis (*2*). Leukotrienes C_4 and D_4 (LTC_4 and LTD_4) have recently been identified as the major active constituents of slow-reacting substance of anaphylaxis (SRS-A) (*3–5*). Both are produced in vitro by the combined activation of human mast cells and pulmonary interstitial mononuclear cells (*6*). Human polymorphonuclear cells convert synthetic leukotriene A_4 to LTC_4 (*7*), which can then be transformed to LTD_4. To seek clues to the precise mechanisms of cardiovascular depression, we evaluated the effects of intracoronary injections of synthetic LTD_4 on sheep coronary artery blood flow and myocardial performance in vivo before and after the systemic administration of an inhibitor of cyclooxygenase and an SRS-A antagonist, and observed the concentrations of coronary sinus thromboxane B_2 (TxB_2) and 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) before and after intracoronary injection of synthetic LTD_4.

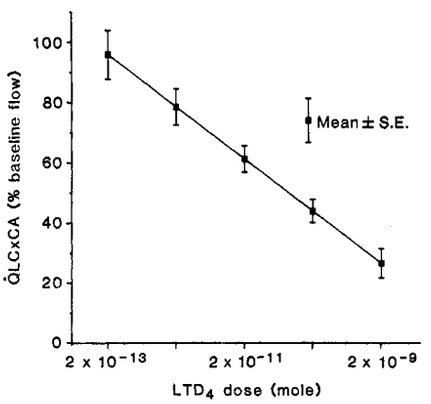

Fig. 1. Inverse log dose response relationship between increasing doses of LTD_4 and LCx coronary artery flow. Left circumflex coronary artery (*LCxCA*) flow is indicated as a percentage of the baseline value.

Eight Suffolk sheep of both sexes weighing 30 to 35 kg were anesthetized and ventilated with 0.5 percent halothane in oxygen. Pulmonary artery and wedge pressure and right ventricular filling pressure were recorded with a flow-directed catheter. Cardiac output was intermittently determined by thermodilution. Systemic arterial pressure, airway pressure, and lead II of the electrocardiogram were continuously recorded. Through a left thoracotomy an electromagnetic flow probe was placed around the main pulmonary artery. An electromagnetic flow probe was positioned around the left circumflex (LCx) coronary artery proximal to the first diagonal branch. The first diagonal branch was cannulated in retrograde fashion with an 0.7-mm (outer diameter) polyethylene catheter and the tip advanced to the confluence of that branch with the LCx coronary artery. A catheter was introduced into the coronary sinus for blood sampling. Left ventricular pressure was measured with a Millar micromanometer tip pressure catheter. The first derivative of left ventricular pressure ($LVdP/dt$) was obtained by electronic differentiation.

In the first group of five sheep, 1 ml of normal saline at 39°C was initially inject-

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