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PROTEIN AND ELECTRORETINOGRAM CHANGES IN THE ALLELES OF THE *norp* A^{P12} *DROSOPHILA* PHOTOTRANSDUCTION MUTANT*

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SUMMARY

The protein differences found in the *norp* A^{P12} phototransduction mutant were studied in six other *norp* A alleles. The two proteins which had altered concentrations in the *norp* A^{P12} mutant were found to have altered concentrations in each of the other alleles. The amount of concentration change varied with the particular allele and appeared related to the reduced visual receptor potentials observed in the mutant. The alleles with the more altered protein concentration exhibited the smaller receptor potentials while alleles with less altered protein concentrations exhibited the larger receptor potentials.

Each of the observable protein subunits in the *Drosophila melanogaster* eye were characterized as to mobility and molecular weight. Fourteen protein subunits were characterized. No mobility changes were detected in any of the eye proteins nor were there any clear concentration changes observed, except for the two proteins altered in all *norp* A alleles.

INTRODUCTION

The use of induced mutant strains has been of great value in clarifying and evaluating biological pathways and mechanisms. In order to apply such techniques to an analysis of the visual system Pak et al. [1] induced and isolated a number of single lesion mutants of *Drosophila melanogaster* which showed abnormalities in their electroretinogram. One of the most interesting mutant groups developed was that of *norp* A (no receptor potential, group A; 6.9 ± 0.1 on X-chromosome [2]). Alleles of *norp* A have electroretinograms in which the receptor potential is either absent or reduced in amplitude [3]. These mutants also exhibit an abnormal zipper-like structure in the photoreceptor membrane [4] but have normal membrane potentials [5]. Similar mutants have been reported by Hotta and Benzer [5] and Heisenberg [6].

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For the *norp* A^{P12} (Pak lab, No. 12; formerly x-12) mutant, we have already reported altered protein concentrations [7]. Analyzing the *Drosophila* eye with sodium dodecylsulfate–acrylamide disc gel electrophoresis [8] we found that in the mutant a protein of molecular weight approx. 51 000 appeared to have increased in concentration while a protein of molecular weight about 46 000 appeared to have decreased in concentration. However, for clarification of the basic mechanism underlying the mutation it is necessary to study additional alleles of the *norp* A^{P12} mutant. Thus one needs to know whether the observed concentration changes occurs in all *norp* A mutants and, if so, whether the change is constant or varies with the particular allele. One is also interested in finding out if any correlation exists between the protein concentration changes and the visual receptor potential amplitudes.

The same concentration effect is observed to varying degrees in all *norp* A alleles investigated. The results from six of these are presented. Moreover, the degree of concentration difference appears related to the amplitude of the mutant visual receptor potential.

MATERIALS AND METHODS

In the disc gel electrophoresis, the 10 % acrylamide gels, electrode buffer, Coomasie blue staining solution, and destaining solutions were prepared according to the procedure of Weber and Osborn [8] except that the gel and electrode buffer contained 1 % (rather than 0.1 %) sodium dodecylsulfate. The sample was electrophoresed at 1.5 mA/tube for 15 min and at 8 mA/tube for 6 h (Buchler No. 3-1750 electrophoresis chamber and Buchler No. 3-1014A constant current/voltage power supply). The gels were stained for over 24 h and destained manually or at 8 mA/tube using a horizontal destainer chamber (Canalco Inc.). They were then scanned with a scanning photometer (Helena Co.) using a broad band 570-nm filter, and the 9–10 areas were determined with a Gelman planimeter using the minimum after band 10 as the baseline. We attempted to separate bands 9 and 10 further by decreasing acrylamide, or electrode buffer concentration, or by using longer running times. Although these alterations separated the main peaks slightly more, increased diffusion resulted in less distinct bands and made interpretation more difficult.

Samples were prepared by placing the live *D. melanogaster* in the dark for 24 h and then in a freezer for at least 3 h. The remaining procedures were then done at normal light levels. Only male flies were used. 200–300 wild type (Oregon-R) and 300 mutant eyes were dissected and washed three times in 99 % acetone. After the final wash the acetone was eliminated by evaporation and the precipitate was dissolved in a degassed 0.3 ml solution of 5 % sodium dodecylsulfate in 0.01 M phosphate buffer (1/20 gel buffer). After the precipitate was completely dissolved, 5 μl of β-mercaptoethanol were added. Finally, the solution was incubated for 20 min at 80 °C.

For application of sample to the gel, 50 μl of sample solution, 25 μl of 5 % sodium dodecylsulfate in 0.01 phosphate buffer, 75 μl of gel buffer, 50 μl of glycerol, and 5 μl of β-mercaptoethanol were combined. In most runs, 5 μl of glyceraldehyde-3-phosphate dehydrogenase, 0.86 mg/ml, M_r 36 000 were added as a standard, and only 20 μl of 5 % sodium dodecylsulfate in 0.01 M phosphate buffer was used. In certain cases, fumarase (M_r 49 000) was added (20 μl, 2.4 mg/ml) and the 5 % sodium dodecylsulfate in 0.01 M phosphate buffer solution reduced appropriately.

Total sample volume was constant (205 μl) for all runs.

Experiments were run on the following seven *norp* A alleles: *norp* A^{P12}, *norp* A^{P13}, *norp* A^{P16}, *norp* A^{P24}, *norp* A^{P41}, *norp* A^{H5} (H-Heisenberg, lab, mutant No. 5; formerly opm 5), and *norp* A^{H44}. In each experiment, wild type flies of the Oregon-R stock [9] were used as a reference. In addition, Oregon-R* flies were tested against Oregon-R flies as a control. Oregon-R* flies are a wild type strain derived from the original Oregon-R stock by selecting for positive phototaxis through a number of generations. Once established, this stock was used in the mutagenesis program from which all the Pak mutants were derived.

Three different experimental regimes were used. For Oregon-R*, *norp* A^{P12}, and *norp* A^{P16}, three experiments were run against Oregon-R. Each of the three experiments consisted of 12 gels, six Oregon-R, and six of either Oregon-R*, *norp* A^{P12}, or *norp* A^{P16}. Of the six gels in each group, one had no added proteins, four contained glyceraldehyde-3-phosphate dehydrogenase, and one contained both glyceraldehyde-3-phosphate dehydrogenase and fumarase. The second experimental regime was conducted with *norp* A^{P13}, *norp* A^{P24}, *norp* A^{H5}, and *norp* A^{H44}. For this group, two experiments of twelve gels each were performed. Two gels were Oregon-R, five were one type of mutant and five were a second mutant. Of these gels, one in each group had no added protein while the rest contained glyceraldehyde-3-phosphate dehydrogenase. The third regime was two experiments of twelve gels, half *norp* A^{P41}, and half Oregon-R. Four gels of each contained glyceraldehyde-3-phosphate dehydrogenase, and two were without added proteins.

The protein mobilities were determined in the following manner. The mobility of glyceraldehyde-3-phosphate dehydrogenase (0.412 ± 0.006) was found using bromophenol blue as the tracking dye. The mobilities of the other standard proteins and of the *Drosophila* proteins were determined by comparing the protein mobility, to that of glycetaldehyde-3-phosphate dehydrogenase. The formula used was:

$$\text{Mobility (x)} = \frac{\text{distance of protein migration}}{\text{distance of glyceraldehyde-3-phosphate dehydrogenase migration}} \times 0.41$$

Band No. 8 with a mobility of 0.24 was used as the standard for band No. 12 which was masked by the glyceraldehyde-3-phosphate dehydrogenase.

RESULTS

The scans of representative sodium dodecylsulfate disc gel electrophoresis runs for all of the *norp* A alleles studied and Oregon-R* are compared with those of Oregon-R in Figs 1–3. Except for apparent concentration differences of bands 9 and 10, there are no other significant differences between the proteins of the wild type Oregon-R eye and those of the mutant eyes. Small concentration differences observed for some of the other bands in Figs 1–3 are not consistent for all of the mutant alleles, nor are they found to persist when additional comparisons are made within the same electrophoresis run or in independent runs. (In a run of 6 wild type and 6 mutants a total of 36 comparisons are possible for all the bands except No. 12). From Figs 1–3, the concentration differences in proteins 9 and 10 are quite clear in

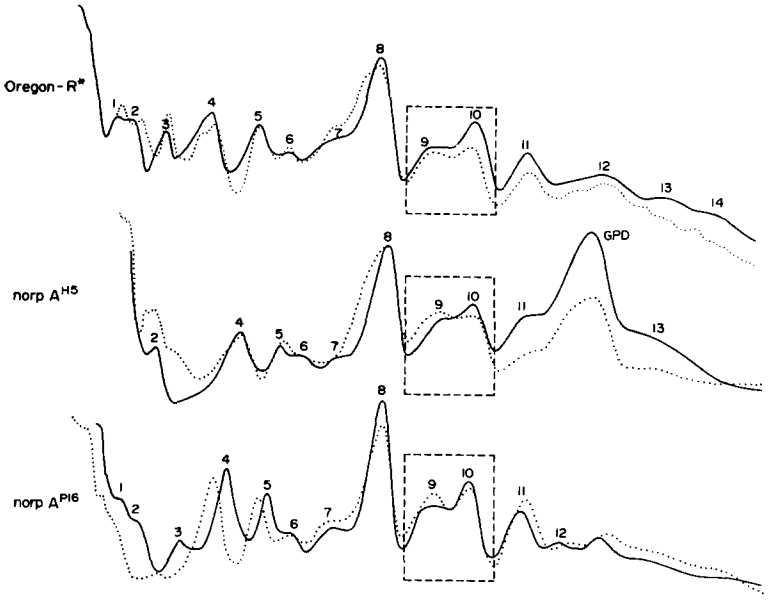

Fig. 1. Representative scans of sodium dodecylsulfate disc gels. Oregon-R (—) versus Oregon-R*, *norp* A^{H5}, *norp* A^{P16} (· · ·). GPD, glyceraldehyde-3-phosphate dehydrogenase.

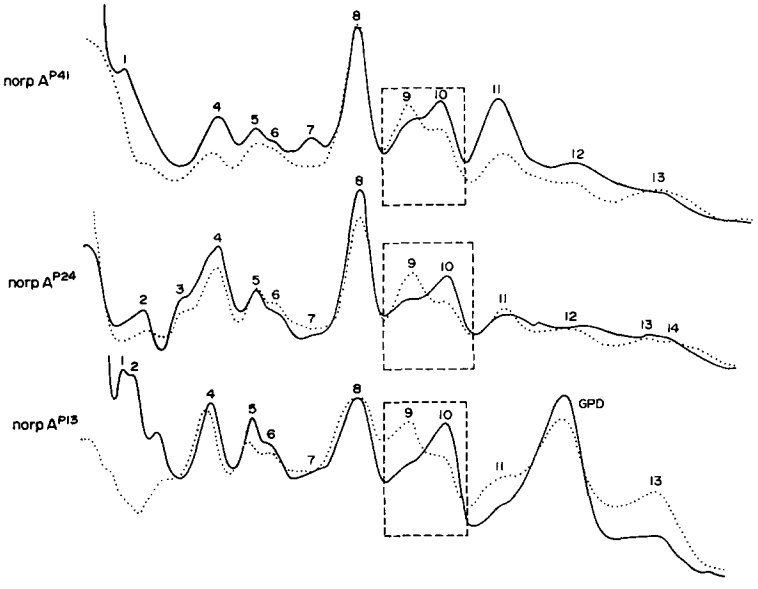

Fig. 2. Representative scans of sodium dodecylsulfate disc gels. Oregon-R (—) versus *norp* A^{P41}, *norp* A^{P24}, *norp* A^{P13} (· · ·). GPD, glyceraldehyde-3-phosphate dehydrogenase.

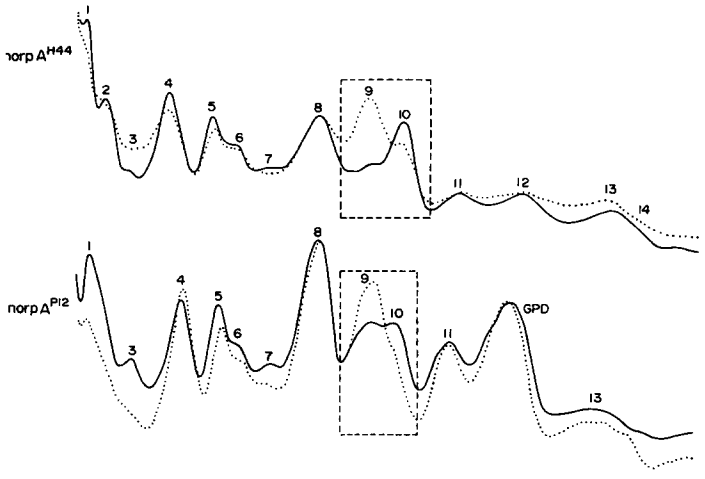

Fig. 3. Representative scans of sodium dodecylsulfate disc gels. Oregon-R (—) versus *norp* A^{P12}, *norp* A^{H44} (· · ·). GPD, glyceraldehyde-3-phosphate dehydrogenase.

mutants *norp* A^{H44} and *norp* A^{P12} (Fig. 3). They are less pronounced, but still relatively prominent, in *norp* A^{P13}, *norp* A^{P24}, and *norp* A^{P41} (Fig. 2). The differences appear to be present but small in *norp* A^{P16} and *norp* A^{H5} (Fig. 1). There does not appear to be any difference between Oregon-R wild type and Oregon-R* (Fig. 1).

To characterize the protein subunits of the *Drosophila* eye, the mobilities and approximate molecular weights of each of the bands were determined and are presented in Table I. The mobilities of the various bands in the mutant and wild type eye appear to be the same (except for a slightly higher mobility for all of the *norp* A^{P13} bands). The detectable concentration changes in bands 9 and 10 and the absence of mobility changes in any of the protein bands, implies that the observable mutant protein differences arose from concentration changes in proteins or subunits which are already present in the wild type eye. In the mutant eyes no new protein bands were detectable and no changes in mobility, indicative of molecular weight changes in the wild type proteins, were observed although unobserved protein changes might be occurring.

The molecular weights of the proteins are also presented in Table I. They were obtained by comparing the mobilities of the protein components with the standard proteins shown in Fig. 4. The affected proteins 9 and 10 have molecular weights of 51 000 and 46 000 respectively.

To quantitate the protein concentration differences in bands 9 and 10, the area of band 10 relative to the total 9–10 area was calculated for each of the electrophoresis runs and the average of all of the runs was taken. At least ten runs were averaged for each strain. The qualitative differences observed in Figs 1–3 are quantitated by this method and the results are presented in Table II.

The degree of change in proteins 9 and 10 appears to be related to the receptor potential amplitude. The maintained, corneal negative component of the *Dipteran* electroretinogram arises almost entirely from the photoreceptors (see a summary by Goldsmith [9]), and this electroretinogram component may therefore be used as a

TABLE I

MOBILITIES AND APPROXIMATE MOLECULAR WEIGHTS OF *DROSOPHILA* EYE PROTEINS

Values ±S.D.

Species	Band No:	1	2	3	4	5	6	7
	Approx. M_r:	240 000	220 000	150 000	140 000	94 000	86 000	76 000
Oregon-R		0.030 (±0.006)	0.043 (±0.009)	0.070 (±0.01)	0.109 (±0.006)	0.145 (±0.005)	0.164 (±0.006)	0.197 (±0.006)
Oregon-R*		0.034 (±0.005)	0.048 (±0.006)	0.074 (±0.008)	0.111 (±0.005)	0.146 (±0.005)	0.166 (±0.004)	0.200 (±0.004)
norp A^{H5}		0.029 (±0.001)	0.045 –	0.075 (±0.001)	0.109 (±0.004)	0.143 (±0.002)	0.162 (±0.001)	0.192 (±0.006)
norp A^{P16}		0.029 (±0.006)	0.044 (±0.006)	0.073 (±0.005)	0.114 (±0.003)	0.147 (±0.003)	0.169 (±0.005)	0.200 (±0.006)
norp A^{P41}		0.028	0.048	0.072	0.104 (±0.005)	0.146 (±0.003)	0.164 (±0.004)	0.189 (±0.001)
norp A^{P24}		0.031 (±0.005)	0.042 (±0.008)	0.067 (±0.010)	0.110 (±0.003)	0.144 (±0.002)	0.162 (±0.002)	0.194 (±0.003)
norp A^{P13}		0.041 (±0.002)	0.069 (±0.002)	0.083 (±0.007)	0.116 (±0.003)	0.149 (±0.003)	0.169 (±0.004)	0.199 (±0.004)
norp A^{P12}		0.026 (±0.003)	0.037 (±0.004)	0.058 (±0.009)	0.105 (±0.006)	0.142 (±0.003)	0.164 (±0.002)	0.192 (±0.003)
norp A^{H44}		0.042 (±0.008)	0.060 (±0.008)	0.079 (±0.010)	0.114 (±0.008)	0.147 (±0.007)	0.166 (±0.008)	0.188 (±0.006)

TABLE I (*continued*)

Species	Band No:	8	9	10	11	12*	13	14
	Approx. M_r:	64 000	51 000	46 000	40 000	37 000	31 500	31 000
Oregon-R		0.236 (±0.005)	0.280 (±0.005)	0.307 (±0.006)	0.356 (±0.006)	0.408 (±0.024)	0.481 (±0.011)	0.505 (±0.014)
Oregon-R*		0.236 (±0.003)	0.279 (±0.005)	0.306 (±0.004)	0.358 (±0.003)	0.432 (±0.031)	0.458 (±0.010)	0.501 (±0.009)
norp A^{H5}		0.232 (±0.002)	0.277 (±0.003)	0.308 (±0.002)	0.355 (±0.002)	0.410 (±0.012)	0.471 (±0.012)	0.510 (±0.007)
norp A^{P16}		0.237 (±0.005)	0.278 (±0.004)	0.308 (±0.003)	0.357 (±0.005)	0.409 (±0.021)	0.482 (±0.016)	0.506 (±0.018)
norp A^{P41}		0.231 (±0.005)	0.277 (±0.003)	0.303 (±0.003)	0.352 (±0.006)	0.405 (±0.021)	0.482 (±0.016)	
norp A^{P24}		0.234 (±0.003)	0.278 (±0.003)	0.306 (±0.001)	0.356 (±0.001)	0.410 (±0.016)	0.476 (±0.009)	0.506 (±0.011)
norp A^{P13}		0.242 (±0.004)	0.283 (±0.002)	0.310 (±0.003)	0.357 (±0.002)	0.394 (±0.008)	0.474 (±0.012)	0.520 (±0.033)
norp A^{P12}		0.236 (±0.002)	0.281 (±0.004)	0.308 (±0.008)	0.357 (±0.003)	0.400 (±0.011)	0.474 (±0.015)	0.505 (±0.012)
norp A^{H44}		0.234 (±0.007)	0.291 (±0.003)	0.307 (±0.004)	0.354 (±0.002)	0.385 (±0.001)	0.471 (±0.017)	0.505 (±0.025)

* Calculated assuming 8 is 0.24.

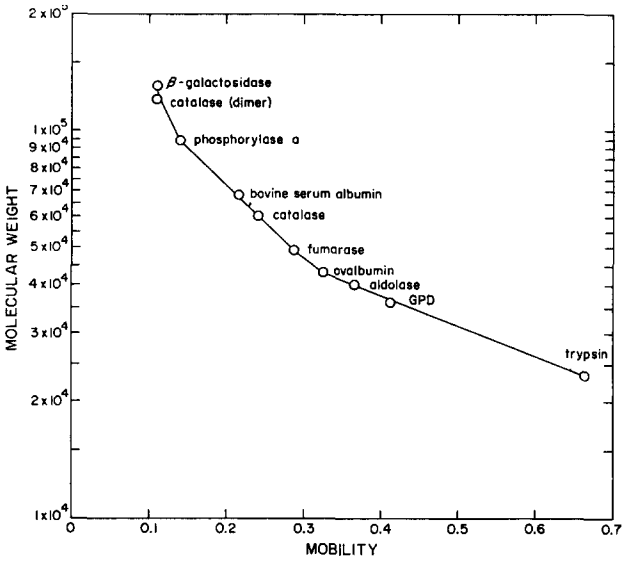

Fig. 4. Mobilities of standard proteins versus molecular weight. Used in calculating molecular weights in Table I. GPD, glyceraldehyde-3-phosphate dehydrogenase.

measure of the receptor potential amplitude. As noted in Table II, the two wild type strains show only small differences in the 9–10 band percentages and have the same electroretinogram amplitudes. All of the *norp* A mutants show reduced percentages of protein no. 10 as well as reduced electroretinogram amplitudes. Among the mutants studied, the two with protein 10 percentages closest to wild type (*norp* A^{H5} and *norp* A^{P16}) have the largest receptor potentials, whereas those with larger changes in the 9–10 proteins have the smaller receptor potentials.

TABLE II

RELATIVE AREAS OF BANDS 9 AND 10 AND ELECTRORETINOGRAMS

Species	% of band 10 in bands 9 and 10 (± S.E.)	Electroretinogram amplitude after 15 min dark adaptation (mV) All flies on (*bw;st*) background
Oregon-R	47 (±2)	20 (±1.4)
Oregon-R*	45 (±2)	20 (±1.4)
norp A^{H5}	40 (±1)	13.7 (±1.1)
norp A^{P16}	38 (±2)	17.7 (±1)
norp A^{P41}	32 (±2)	0
norp A^{P24}	28 (±3)	0
norp A^{P13}	24 (±1)	1.4 (±0.3)
norp A^{P12}	19 (±2)	2 (±0.2)
norp A^{H44}	13 (±1)	0

DISCUSSION

All of the *norp* A visual mutants exhibit differences in bands 9 and 10 when analyzed by sodium dodecylsulfate–acrylamide disc gel electrophoresis. The extent of these differences is a function of the particular mutant (Figs 1–3). Thus the percent of protein 10 in the 9–10 area varies from 47 % in the wild type and 40 % in *norp* A^{H5}, the least affected mutant allele, to only 13 % in the most affected allele *norp* A^{H44} (Table II). Moreover, no mobility differences are observed in any of the protein bands (Table I). Therefore, the most straightforward interpretation of the data is that concentrations of proteins 9 and 10 have changed in the mutant, with an increase in protein 9 and a decrease in protein 10. For example, in a single electrophoretic run of six *norp* A^{P12} and six wild type eyes, the average areas of proteins 9 and 10 in the wild type (corrected to a standard glyceraldehyde-3-phosphate dehydrogenase area of 10 $inch^2$ were 0.17 $inch^2$ and 0.15 $inch^2$, respectively. In the *norp* A^{P12} mutant, the area of protein 9 went up to 0.47 $inch^2$ and protein 10 down to 0.09 $inch^2$. However, because of the difficulties involved in this type of analysis, it does not seem possible to eliminate completely some alternative explanations, such as increases in the concentration of only protein 9, production of a new protein electrophoretically close to protein 9, or a change in mobility of another protein which is originally buried in the 9–10 area.

From Table II, it can be seen that the degree of change in proteins 9 and 10 is related to the degree of reduction in electroretinogram amplitude. In general, the mutants with 9–10 areas closest to that of wild type, *norp* A^{H5} and *norp* A^{P16}, have the largest electroretinograms while the remaining mutants have little or no electroretinograms. The electroretinograms listed in Table II were obtained from flies placed on a white eye background provided by a combination of eye color mutations brown (*bw*) and scarlet (*st*) [9]. This removes the screening pigments in the eye without affecting the visual pigment(s) and thus increases the amount of light reaching the visual pigment(s). If the electroretinograms are obtained from flies having normal screening pigments, the abrupt transition in electroretinogram amplitude between *norp* A^{P16} and *norp* A^{P41} becomes even more striking. The electroretinograms of Oregon-R and R*, *norp* A^{H5}, and *norp* A^{P16} remain large, but no electroretinogram at all can be elicited from the rest of the mutants listed in Table II. It would appear that a critical chemical change has taken place, and one may suggest that this change is related to the increased concentration of protein 9, decreased concentration of protein 10, or a combination of both.

In addition to this main process, there may be a secondary process which also affects the amplitude of the electroretinogram, though to a lesser extent. It may be seen in Table II that small electroretinogram potentials are obtained from *norp* A^{P12} and *norp* A^{P13}, even though they lie below the presumed critical point of protein 9–10 changes and even though chemically less affected alleles, *norp* A^{P41} and *norp* A^{P24}, display no electroretinograms. One possible explanation for this anomaly might be that a secondary process not directly related to the 9–10 changes is operational in *norp* A^{P13} and *norp* A^{P12}, such as effects on different populations of cells. Within the framework of this hypothesis, the main process discussed above presumably is blocked in all mutant alleles listed below *norp* A^{P16} in Table II. The secondary process, however, is operational in *norp* A^{P13} and *norp* A^{P12}, while this, too, is

blocked in the other three mutant alleles (*norp* A^{P41}, *norp* A^{P24}, and *norp* A^{H44}).

The identity of the altered proteins 9 and 10 as well as the other observed protein bands is not yet known. We list below some of the proteins found in the frog retina [10], with corresponding molecular weights from various sources: ATPase, M_r 50 000, (mitochondrial [11]); glucose-6-phosphate dehydrogenase, M_r 51 300, (human [12]) M_r 63 000 (mammary [13]); malate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, and lactate dehydrogenase, M_r 35 000–36 000 [14–16], squid rhodopsin, M_r 70 000 [17] or 49 000 [18], and cattle rhodopsin, M_r 35 000 [19].

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REFERENCES

1 Pak, W. L., Grossfield, J. and White, N. V. (1969) Nature 222, 351–354
2 Pak, W. L., Grossfield, J. and Arnold, K. S. (1970) Nature 227, 518–520
3 Alawi, A. A. (1972) Ph. D. Thesis. Purdue University
4 Alawi, A. A., Jennings, V., Grossfield, J. and Pak, W. L. (1972) in The Visual System, (Arden, G. B., ed.), Vol. 24, pp. 1–19, Plenum Press, New York
5 Hotta, Y. and Benzer, S. (1970) Proc. Natl. Acad. Sci. 67, 1156–1163
6 Heisenberg, M. (1971) Drosophila Inform. Serv. 46, 68
7 Ostroy, S. E. and Pak, W. L. (1973) Nat. New Biol. 243, 120–121
8 Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412
9 Lindsley, D. L. and Grell, E. H. (1968) Carnegie Inst. Wash. Publ. No. 627
10 Lolley, R. N. and Hess, H. (1969) J. Cell Physiol. 73, 9–24
11 Senior, A. E. and Brooks, J. C. (1971) Fed. Eur. Biochem. Soc. 17, 327–329
12 Bonsignore, A., Cancedda, R., Lorenzoni, I., Consulich, M. E. and DeFlora, A. (1971) Biochem. Biophys. Res. Commun. 43, 94–101
13 Levy, H. R., Raineri, R. R. and Nevaldine, B. H. (1966) J. Biol. Chem. 241, 2181–2187
14 Dévényi, T., Rogers, S. J. and Wolfe, R. G. (1966) Nature 210, 489–491
15 Harrington, W. F. and Karr, G. M. (1965) J. Mol. Biol. 13, 885–893
16 Castellino, F. J. and Barker, R. (1968) Biochemistry 7, 2207–2217
17 Fager, R. S., Kimbel, R. L. and Abrahamson, E. W. (1971) Biophys. Soc. Abstr. 11, 45a
18 Hagins, F. M. (1973) J. Biol. Chem. 248, 3298–3304
19 Abrahamson, E. W. and Fager, R. S. (1973) in Curr. Top. Bioenerg. (Sanadi, D. R., and Packer, L., ed.), Vol. 5, pp. 125–200, Academic Press, New York