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Gene encoding cytoskeletal proteins in *Drosophila* rhabdomeres

(photoreceptor/gene dosage)

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ABSTRACT **The *ninaC* gene is one of eight *nina* (neither inactivation nor afterpotential) genes identified from mutations that drastically reduce the amount of rhodopsin in the compound eye of *Drosophila melanogaster*. The gene has been cytogenetically localized to the 27E–28B region of the second chromosome. NaDodSO$_4$/PAGE analysis of eye proteins of flies carrying one, two, or three copies of the *ninaC* region shows that two eye-specific proteins of molecular weight 170,000 and 130,000 display a strong dependence on the dosage of the *ninaC* gene, although the dependence is evident only when the dosage is decreased and not when it is increased. All mutations in the *ninaC* gene studied to date have pronounced effects on these two polypeptides. These results suggest that the *ninaC* locus encodes these two polypeptides. Ultrastructural studies show that the polypeptides encoded by *ninaC* are very likely to be important components of the cytoskeletal structure of rhabdomeral microvilli.**

Among the many *Drosophila melanogaster* mutants that have been isolated in this laboratory on the basis of their defects in light-evoked electrical responses of the eye (1, 2) are those with greatly reduced amounts of the visual pigment, rhodopsin (2–4). These mutants were isolated on the basis of their defect in the prolonged depolarizing afterpotential (PDA) produced by a bright blue stimulus that photoconverts a substantial net amount of rhodopsin to metarhodopsin (Fig. 1) (5–7). In mutants with greatly reduced amounts of rhodopsin, the PDA is either very much reduced in size or totally absent (Fig. 1). The inactivation of the photoreceptors, which accompanies the afterpotential, is also concomitantly reduced. Because of these phenotypes, these mutants have been named *nina* (neither inactivation nor afterpotential).

The *nina* mutants identified to date fall into eight complementation groups, *ninaA–H*. Presumably, many *nina* genes have been identified because a reduction in the amount of visual pigment can occur in a number of different ways. For example, a defect in the gene encoding opsin (e.g., *ninaE*; refs. 8 and 9) or in the genes involved in the formation of chromophore (e.g., *ninaB* and *-D*; ref. 4) is expected to lead to a reduction in the amount of rhodopsin.

Other types of mutations that could reduce the rhodopsin content include those that cause defects in the structure of the rhabdomeral microvilli. The rhabdomere is a cylindrically shaped, specialized photoreceptive organelle of invertebrate photoreceptors and consists of tightly packed microvilli projecting from the cell body of the photoreceptor. The microvilli are arranged in parallel with their axes perpendicular to the optic axis of the rhabdomere. Rhabdomeral microvilli contain most, though not all (10), of the rhodopsin molecules in the photoreceptor as intrinsic membrane proteins. One would expect that any defect in the structure of the rhabdomere would also lead to a reduced rhodopsin content. In this report, we present evidence that one of our *nina* genes, *ninaC*, encodes polypeptides that contribute to the cytoskeletal structure of rhabdomeral microvilli of fly photoreceptors.

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MATERIALS AND METHODS

Flies. All *nina* mutants of *Drosophila melanogaster* used in this work were recovered in chemical mutagenesis of wild-type flies of the Oregon-R strain, as described (2). A total of 11 independently arising *ninaC* mutations have been isolated. The control wild-type flies were also of the same strain. They were maintained in a 12 hr light/12 hr dark illumination cycle at 25°C. Only young flies, 2 to 5 days post-eclosion, were used in this work. Unless noted otherwise, all flies carried the mutation white (*w*) and consequently lacked the screening pigments in the eye. Without this precaution, the red screening pigments tended to filter out the blue wavelengths that are needed to generate the PDA and also interfered with microspectrophotometric measurements of rhodopsin levels.

Genetic Analysis. Recombination mapping of *ninaC* was carried out using multiply marked second chromosomes, *S Sp Bl L bw^D* and *S Sp Bl L^2 Px*, obtained from the stock center at the California Institute of Technology. Cytogenetic mapping was performed by testing for complementation with segmental aneuploid deficiencies constructed using the stocks and techniques described by Lindsley *et al.* (11). Two synthetic deficiencies were constructed for this purpose, one using the translocation stocks *T(Y;2)R147* and *T(Y;2)R50* (designated *Df(2L)R147-R50*) and the other using *T(Y;2)R147* and *T(Y;2)H52* (designated *Df(2L)R147-H52*). In both kinds of mapping, the electroretinogram (ERG) was used to assay for the mutant phenotype. The second synthetic deficiency was also used to test for gene-dosage dependence. In addition, a duplication for the region 26F to 28B4-C1, designated *Dp(2L)H52-J136*, was constructed using the translocation stocks *T(Y;2)J136* and *T(Y;2)H52*. To minimize differences in genetic background, flies of the translocation stock *T(Y;2)-H52* were used as controls. None of the aneuploid flies constructed nor the control flies had Bar-shaped eyes (*B^s*) because we used translocation stocks from which the *B^s* marker was spontaneously lost. The autosomal breakpoints of all these translocations, given in ref. 11, were reexamined by means of the standard technique for salivary chromosome analysis (12). Unlike the other flies used, flies of the translocation and deficiency stocks did not carry the mutation white.

Histology. Heads dissected from anesthetized flies were split in half and fixed in 2% paraformaldehyde/2%

Abbreviations: ERG, electroretinogram; PDA, prolonged depolarizing afterpotential.

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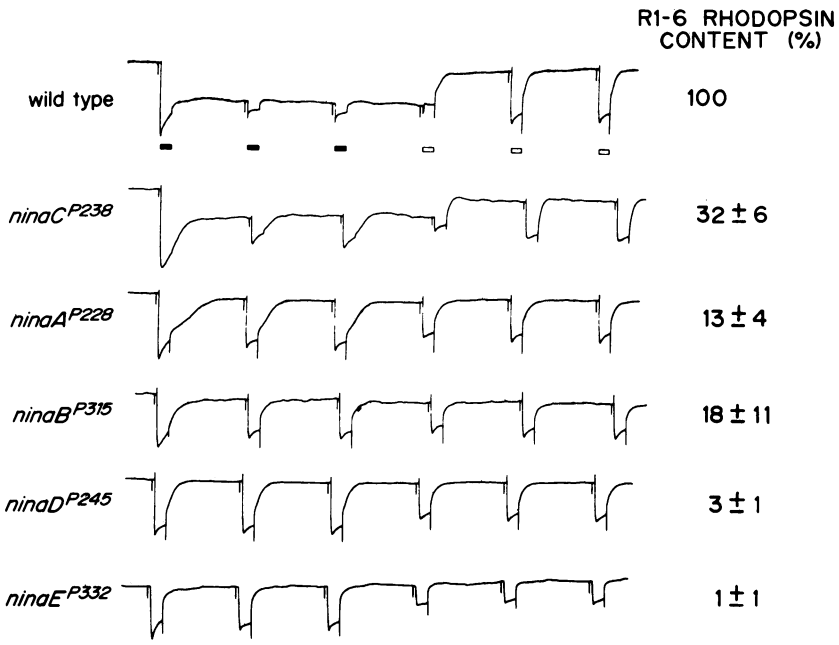

FIG. 1. ERG recordings obtained from a wild-type fly and one representative mutant from each of five *nina* complementation groups. The records are arranged from top to bottom in increasing order of severity of the *nina* phenotype. Three blue stimuli (solid bars) followed by three orange stimuli (open bars) are presented sequentially at 30-sec intervals. The stimulus duration was 4 sec. A 5-mV calibration pulse just precedes each response. To the right of each ERG trace is an approximate rhodopsin content in the R1–R6 photoreceptors of that class of flies, determined by *in vivo* microspectrophotometry and given as a fraction of the wild-type level. (Modified from figure 1 and table 2 of ref. 4; reproduced with permission from Cambridge University Press.)

gluteraldehyde/0.1 M phosphate buffer, pH 7.4, on ice for 3–5 hr. They were postfixed in 2% osmium tetroxide for 1–2 hr on ice, dehydrated in an ethanol series, substituted with propylene oxide, and embedded in Epon 812. Sections were cut with a diamond knife using a Sorvall MT2-B Porter Blum ultramicrotome. They were then stained with uranyl acetate, poststained with Reynold's lead citrate, and examined on a Phillips EM300 electron microscope at 60 kV.

ERG Recording and *in Vivo* Microspectrophotometry. Techniques for ERG recording (13) and for *in vivo* microspectrophotometry (14) have been described in detail.

Electrophoretic Analyses of Retina-Specific Polypeptides. A method described by Fujita and Hotta (15) was used to obtain tissue-specific samples of fly heads. Flies were frozen in a test tube at liquid nitrogen temperature and dismembered by Vortex mixing. After 2–3 days in acetone at –20°C, the acetone was removed by filtering followed by evaporation. The compound eyes were then dissected from isolated heads, using a dissecting needle under a stereo microscope. For some purposes, the isolated compound eye was further dissected into the cornea and the photoreceptor layer. For electrophoresis, a tissue sample from 10 or 15 flies was homogenized in 30 μl of NaDodSO$_4$/PAGE lysis buffer (2% sodium dodecyl sulfate/5% 2-mercaptoethanol/62.5 mM Tris·HCl, pH 6.8) and centrifuged at 4000 × *g* for 15 min. The supernatant was then subjected to discontinuous NaDodSO$_4$/PAGE in 7.5% or 10% gels. Gels were stained with either Coomassie blue or silver.

To test for gene-dosage dependence, the amounts of the polypeptides that are altered in the mutants (designated B1–B5, see *Results* and Fig. 4) were determined by densitometric scanning of Coomassie blue-stained gels or their photographic negatives. The linearity between the amount of protein and the measured density was tested by scanning the photographic negative of a gel loaded with known amounts of a standard protein, bovine serum albumin.

The density of B1–B4 polypeptides was scanned in one dimension, using an LKB model SLSD-11 soft-laser densitometric scanner, in a direction perpendicular to the lanes, following a given polypeptide from one lane to the next. For each polypeptide, the densitometric reading for control flies carrying two copies of *ninaC*$^+$ was taken as 100%, and readings of B1 and B4 in homozygous *ninaC*^{P230} or *ninaC*^{P235} mutant flies were taken as background. B1 and B4 polypeptides were consistently missing in these mutants (see Fig. 5). In most gels scanned, it was difficult to obtain reliable readings of B5 because the amounts of this polypeptide present in the gel were so small. For this reason, the analysis was carried out only on B1–B4 polypeptides.

RESULTS

Electrophysiology. Fig. 1 compares the electroretinograms (ERGs) evoked by successive blue and orange stimuli from a wild-type fly (top trace) and one mutant from each of the five *nina* complementation groups, *ninaA*, *-B*, *-C*, *-D*, and *-E*. In wild-type flies, a blue stimulus evokes a very prominent afterpotential that persists long after the stimulus is turned off (Fig. 1, top trace). In addition, the responses evoked by the second and third blue stimuli are small because the first blue stimulus inactivates the major class of photoreceptors, R1–6, and only the minor photoreceptors, R7 and R8, contribute to these responses (16). In the mutants, the afterpotential is small or absent. In addition, the responses to the second and third blue stimuli are larger (Fig. 1), because the mutations reduce or remove the inactivation of R1–6 photoreceptors by the first blue stimulus, and R1–6 photoreceptors also contribute to these responses. The *ninaC* ERG (Fig. 1, second trace) differs from ERGs of other *nina* mutants in that substantial amounts of both the afterpotential and the inactivation still remain in the ERG.

Shown on the right-hand side of Fig. 1 are relative rhodopsin levels in R1–6 photoreceptors determined by *in vivo* spectrophotometry of single fly eyes (4). It may be seen that, in parallel with the larger PDA, the *ninaC* mutant has substantially more R1–6 rhodopsin than any other *nina* mutant. More recent measurements on *ninaC*^{P221} and *ninaC*^{P238} (2–4 days post-eclosion) yielded 27 ± 9% and 41 ± 7% of wild-type level, respectively, consistent with earlier measurements (L. L. Randall, personal communication).

Genetics and Cytogenetics. Attempts to map the *ninaC* mutation using a multiply marked second chromosome revealed that all chromosomes bearing Sternopleural (*Sp*) [mapping at 2-22.0 (17)] that we tested failed to complement *ninaC*. However, none of our *ninaC* mutants displayed the morphological phenotype of *Sp*, suggesting that the mutation *Sp* affects more than one locus. In an attempt to separate a *ninaC* allele from the *Sp*-bearing chromosomes, we examined 19 recombinant chromosomes with crossovers in the interval between *S* [mapping at 2-1.3 (17)] and *Sp*, and 27 others with

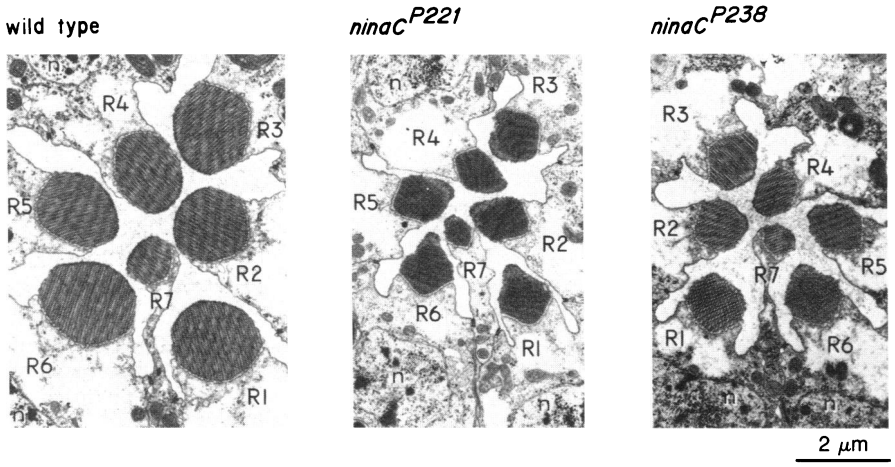

FIG. 2. Electron micrographs comparing the rhabdomeres of wild type, *ninaC*^{P221}, and *ninaC*^{P238}. Because the size of a rhabdomere varies along its length, the comparison is made on transverse sections in the distal retina at the level of the nuclei of R1–6 photoreceptors. Note that both alleles of *ninaC* significantly reduce the cross-sectional diameters of rhabdomeres of R1–6, as well as R7, photoreceptors. n, Nucleus.

crossovers in the interval between *Sp* and *Bl* [mapping at 2-54.8 (17)]. None of these recombination events separated *ninaC* from *Sp*.

Segmental aneuploid deficiencies, *Df(2L)R147-R50* and *Df(2L)R147-H52*, both uncovered the *ninaC* mutation. The previously reported autosomal breakpoints of *R147, R50*, and *H52* are 27E, 28B, and 27E, respectively (11). Our own analysis, however, showed the autosomal breakpoint of *H52* to be 28B4-C1. We, therefore, place *ninaC* in the chromosome region 27E–28B.

Electron Microscopy. Fig. 2 compares electron micrographs of transverse sections through the ommatidia of wild type and the mutants *ninaC*^{P221} and *ninaC*^{P238}. Because the cross-sectional area of a rhabdomere varies along its length, these comparisons were made at the level of the R1–6 cell nucleus. The most noticeable difference between wild type and the mutants seen in these micrographs is the smaller cross-sectional areas of *ninaC* rhabdomeres.

Shown at higher magnification in Fig. 3 are cross-sectional profiles of R1–6 rhabdomeral microvilli of wild type, *ninaC*^{P221}, *ninaC*^{P238}, and *ninaE*^{P318}. A striking difference between the microvilli of the two *ninaC* mutants and those of either wild-type flies or the *ninaE*^{P318} mutant is that an electron-dense core conspicuously present in each microvillus of wild type and *ninaE*^{P318} is either very faint or missing in both *ninaC* mutants examined.

Biochemistry. One-dimensional NaDodSO$_4$/PAGE was carried out to compare retina-specific polypeptides of *ninaC* mutants with those of wild type. Among the slowly migrating polypeptides separated by NaDodSO$_4$/PAGE, five major polypeptide bands, labeled B1–B5, were retina-specific (Fig. 4). These polypeptides, ranging in molecular weight from 170,000 (B1) to 120,000 (B5), were identified to be retina-specific because they appeared in electrophoretic patterns of compound eye (lane 2) and eye-minus-cornea (lane 3) samples but not in those of cornea (lane 4) and head-minus-eyes (lane 5) samples. In a *ninaC* mutant, most of these five bands are either missing or reduced in density (lane 6).

Systematic electrophoretic studies were carried out on nine *ninaC* alleles. Presented in Fig. 5 are a representative set of results (Fig. 5*a*) and a schematic diagram based on many such experiments (Fig. 5*b*). All nine *ninaC* alleles had pronounced effects on either B1 or both B1 and B4. In addition, these mutations reduced the amount of the remaining polypeptides (B2, B3, and B5). On the basis of their effects on B1 and B4, the nine *ninaC* alleles were classified into three groups: group I, consisting of *P221, P238, P239*, and *P240*; group II, consisting of *P216, P230, P235*, and *P262*; and group III, consisting of the *P225* allele (Fig. 5*b*). The mutations in group I eliminated the B1 band but had no effect on B4, the mutations in group II eliminated both the B1 and B4 polypeptides, and the *P225* mutation eliminated B1 and increased the mobility of B4. [Two alternative interpretations of the electrophoretic pattern of *P225*—(*i*) that the mutation eliminates B4 and increases the mobility of B1 and (*ii*) that the mutation eliminates both B1 and B4 and increases the amount of B5—cannot be excluded on the basis of our data alone.] In addition to their effects on B1 and B4, all nine *ninaC* alleles reduced the amounts of B2, B3, and B5 polypeptides.

Gene-Dosage Dependence. The effects of altering the *ninaC*

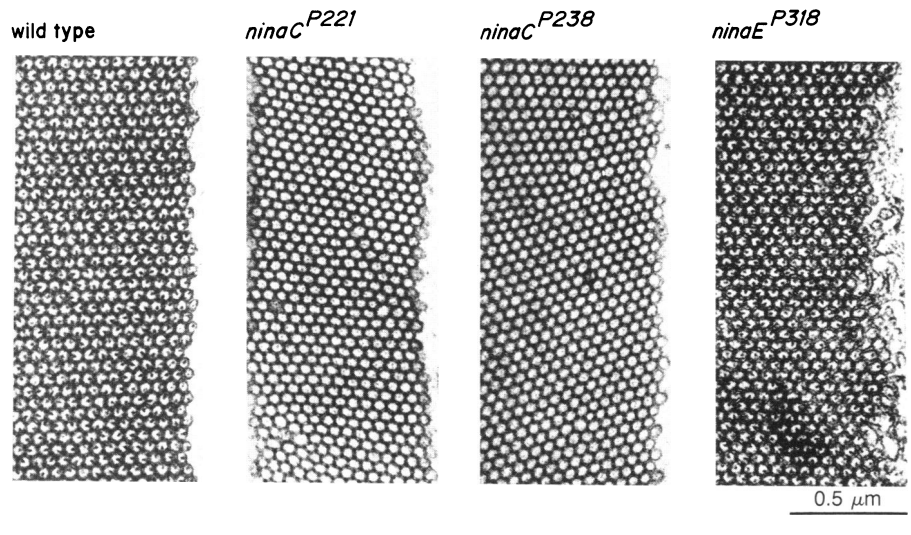

FIG. 3. Electron micrographs of transverse sections through rhabdomeric microvilli of R1–6 photoreceptors of wild type, *ninaC*^{P221}, *ninaC*^{P238}, and *ninaE*^{P318}. Note the prominent electron-dense axial cores in the microvilli of wild type and *ninaE*^{P318}, but not in those of the two *ninaC* mutants. The class of photoreceptors from which these examples were taken are R6, R2, R5, and R4 for wild type, *ninaC*^{P221}, *ninaC*^{P238}, and *ninaE*^{P318}, respectively.

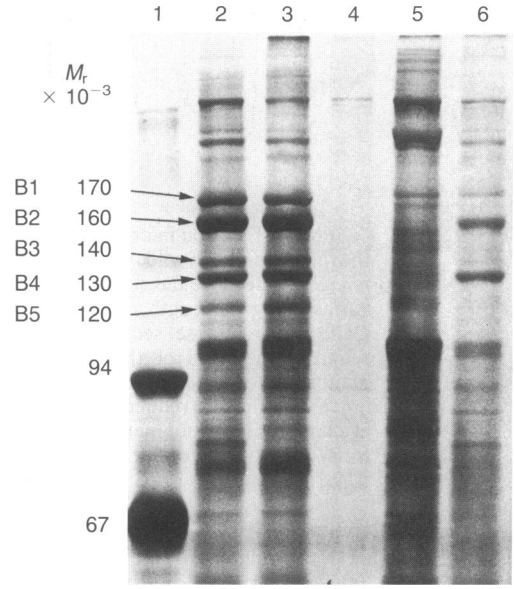

FIG. 4. One-dimensional polyacrylamide gel electrophoretic patterns showing the specificity of the five polypeptides B1–B5 for the retina and the effects of a *ninaC* mutation on these polypeptides. Samples for lanes 2–5, obtained from wild-type flies, were as follows: compound eyes (lane 2), compound eyes from which the corneas had been removed (lane 3), corneas (lane 4), and heads from which the compound eyes had been removed (lane 5). The sample in lane 6 was from compound eyes of the *ninaC*^{P238} mutant. The photoreceptor-layer-specific polypeptides B1–B5 are seen in lanes 2 and 3, but not in lanes 4 and 5. They are either reduced in amount or absent in lane 6. Lane 1 shows the molecular weight markers phosphorylase *b* (M_r 94,000) and bovine serum albumin (M_r 67,000).

gene dosage on the amount of B1–B5 were examined in two ways. In one set of experiments, we compared NaDodSO$_4$/PAGE profiles of compound-eye preparations of *ninaC*^{P230} homozygotes and heterozygotes with that of wild-type eye preparation. The *ninaC*^{P230} allele was chosen because it eliminates both B1 and B4 when homozygous (group II in Fig. 5*b*). The results of densitometric scanning based on five sets of electrophoretic profiles are presented in Table 1. The densities of B1 and B4 were approximately 57% and 53% of the wild-type density, respectively, in heterozygotes and 0%

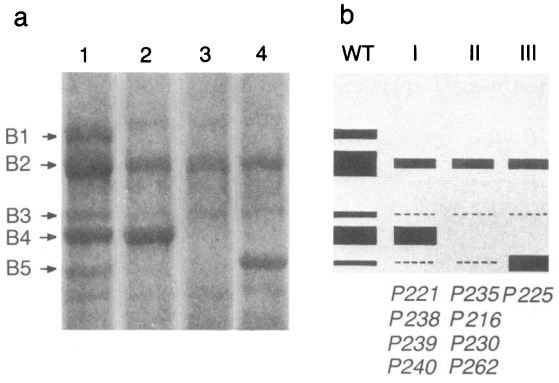

FIG. 5. Classification of *ninaC* mutants into three groups on the basis of their electrophoretic phenotype. (*a*) Examples of NaDodSO$_4$/PAGE patterns of wild type (lane 1) and three *ninaC* mutants each representing a group: *P221* (lane 2), *P235* (lane 3), and *P225* (lane 4). (*b*) A schematic representation of the electrophoretic phenotypes of wild type (WT) and mutants in the three groups (I–III). The *ninaC* alleles in the three groups differ from each other in their effects on polypeptides B1 and B4. Those in group I eliminate B1, those in group II eliminate both polypeptides, and the sole allele in the third group eliminates B1 and alters the mobility of B4.

Table 1. Semidominance of *ninaC*^{P230} for the amount of B1 and B4

Genotype	Relative amount of polypeptide, % +/+ (mean ± SD) B1 (n = 5)	B2 (n = 5)	B3 (n = 4)	B4 (n = 4)
w; *ninaC*^{P230}/*ninaC*^{P230}	0	52 ± 14	58 ± 36	0
w; *ninaC*^{P230} /+	57 ± 5	83 ± 12	90 ± 9	53 ± 4
w; +/+	100	100	100	100

in homozygotes (Table 1). The average densities of B2 and B3 in heterozygotes also tended to be lower than those of wild type, but the amount of reduction in these polypeptides was small (Table 1). Thus, *ninaC*^{P230} was semidominant in its effect on B1 and B4 but not on B2 or B3.

In another set of experiments, flies carrying one, two, or three copies of the *ninaC* region (see *Materials and Methods*) were analyzed for the amount of B1–B4 polypeptides. The densitometric results based on five sets of electrophoretic data are shown in Table 2. For each polypeptide, the results were normalized to the density of the polypeptide in the control flies, *T(Y;2)H52*/+, with two copies of *ninaC*$^+$. Flies carrying one copy of the *ninaC* region displayed 40% and 50% decreases in B1 and B4, respectively, but only a slight decrease in the other two polypeptides, B2 and B3. Inexplicably, the amount of all four polypeptides was somewhat reduced in flies carrying a duplication of the *ninaC* region. The reason for this decrease is not clear. In any event, there was no indication that any of the four polypeptides has any strong dependence on increased *ninaC*$^+$ dosage.

DISCUSSION

To address the question of whether any of the five polypeptides B1–B5 are encoded by the *ninaC* gene, we examined the dependence of these polypeptides on the *ninaC* dosage.

A well-known characteristic of eukaryotic enzyme genes is that the amount of the protein product encoded by a gene depends on the dosage of that gene (18). As shown in Tables 1 and 2, the amount of B1 and B4 polypeptides is reduced to about one-half of their normal level in both *ninaC*^{P230} heterozygotes and deficiency heterozygotes carrying one copy of the normal *ninaC* gene. The polypeptides B2 and B3 also tend to be reduced in amount in these heterozygotes, but the magnitude of decrease is much smaller than for B1 or B4 (Tables 1 and 2). Flies carrying a duplication of the *ninaC* region do not show any increase in the amount of either B1 or B4 (Table 2). Thus, of the five retina-specific polypeptides in question, only B1 and B4 show a clean gene-dosage dependence, and only when the dosage is decreased.

Failure to observe increased amounts of B1 and B4 when the *ninaC*$^+$ dosage is increased may be related to the fact that these polypeptides normally occupy specific or restricted sites in or on the membrane. For example, if all membrane sites for the polypeptides are fully occupied in wild-type flies, further increase in gene dosage will not increase the amount of the polypeptides. Thus, the results of our experiments lead to the conclusion that the *ninaC* gene very likely encodes both B1 and B4 polypeptides.

This conclusion is completely supported by the recent work of C. Montell and G. M. Rubin (personal communication), who found that one of the head-specific genomic clones isolated previously by Levy *et al.* (19) harbors the *ninaC* gene. They found that the cloned DNA, when introduced into the genome of a *ninaC* mutant by P-factor-mediated germ-line transformation, complemented the mutant phenotype (20). They then generated a β-galactosidase-*ninaC* fusion protein (21) and raised antiserum against this protein in a mouse. Immunoblots of electrophoretically fractionated wild-type

Table 2. Gene-dosage dependence of polypeptides B1–B4

Genotype	Copies of *ninaC*$^+$	Relative amount of polypeptide, % (mean ± SD, n = 5)			
		B1	B2	B3	B4
Df(2L)R147-H52/+	1	60 ± 13	85 ± 10	97 ± 13	50 ± 14
T(Y;2)H52/+	2	100	100	100	100
w; Dp(2L)H52-J136/+	3	83 ± 20	85 ± 6	81 ± 9	77 ± 27

and *ninaC* mutant head proteins stained with the antiserum showed that the antiserum recognizes only B1 and B4 polypeptides.

Ultrastructural studies illustrated in Fig. 2 show that, in comparison to other *nina* mutations, the *ninaC* mutations have a disproportionately large effect on the structure of rhabdomeres for a given decrease in the rhodopsin content. The results thus suggest that the reduction in rhodopsin content in *ninaC* mutants is due primarily to the reduced rhabdomeric volume.

A clue to the relationship between the polypeptides encoded by the *ninaC* gene and the structure of the rhabdomere is found in Fig. 3. An electron-dense core present within each rhabdomeral microvillus of wild-type and *ninaE* flies is notably reduced in density or missing altogether in *ninaC* mutants (Fig. 3). Saibil (22) and Blest *et al.* (23) have shown that these densities are associated with the cytoskeleton of the microvillus. According to Blest *et al.* (23), the cytoskeleton of each microvillus consists of an axial filament of 6- to 11-nm diameter running the length of the microvillus and surrounded by amorphous material and a series of side arms linking the axial complex to the plasma membrane of the microvillus. They have also noted that the microvillar cytoskeleton is extremely sensitive to fixation procedures (23). Although we made no special effort to preserve the cytoskeleton, the difference in the microvillar axial structures seen in the *ninaC* mutants and controls was both clear-cut and consistent, ruling out the possibility of a histological artifact. Moreover, the missing or degraded axial densities cannot be a secondary consequence of rhodopsin depletion because they are present in R1–6 rhabdomeres of *ninaE*^{P318}, which contain <10% the amount of rhodopsin in R1–6 rhabdomeres of *ninaC* (25). The results thus strongly suggest that the *ninaC* protein products, B1 and B4, are components of the cytoskeleton of rhabdomeral microvilli, most probably of the microvillar axial filament. Thus, a defect in the *ninaC* gene would result in the deterioration of the rhabdomere.

The B1 and B4 polypeptides were first identified in NaDodSO$_4$/PAGE as part of a set of photoreceptor-layer-specific polypeptides. Electron microscopy of *ninaC* mutants has shown that morphological defects are largely confined to the rhabdomeres of photoreceptors. Both these results suggest that the *ninaC* gene is expressed specifically in photoreceptors. The nature of the proteins that form the rhabdomeral cytoskeleton has not been identified (see ref. 23). Moreover, the molecular genetics of cytoskeletal proteins of nervous tissues has not been explored extensively (24). Thus, the identification of a gene that apparently contributes to the cytoskeleton of a nervous tissue should prove important in the understanding of structural proteins of nervous tissues in general.

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1. Pak, W. L. (1975) in *Handbook of Genetics*, ed. King, R. C. (Plenum, New York), Vol. 3, pp. 703–733.
2. Pak, W. L. (1979) in *Neurogenetics: Genetic Approaches to the Nervous System*, ed. Breakefield, X. O. (Elsevier/North-Holland, New York), pp. 67–99.
3. Pak, W. L., Conrad, S. K., Kremer, N. E., Larrivee, D. C., Schinz, R. H. & Wong, F. (1980) in *Development and Neurobiology of Drosophila*, eds. Siddiqi, O., Babu, P., Hall, L. M. & Hall, J. C. (Plenum, New York), pp. 331–346.
4. Stephenson, R. S., O'Tousa, J., Scavarda, N. J., Randall, L. L. & Pak, W. L. (1983) in *The Biology of Photoreception*, eds. Cosens, D. J. & Vince-Price, D. (Cambridge Univ. Press, Cambridge, England), pp. 477–501.
5. Cosens, D. & Briscoe, D. (1972) *J. Insect Physiol.* **18**, 627–632.
6. Hillman, P., Hochstein, S. & Minke, B. (1972) *Science* **175**, 1486–1488.
7. Brown, H. M. & Cornwall, M. C. (1975) *J. Physiol. (London)* **248**, 555–578.
8. O'Tousa, J. E., Baehr, W., Martin, R. L., Hirsh, J., Pak, W. L. & Applebury, M. L. (1985) *Cell* **40**, 839–850.
9. Zuker, C. S., Cowman, A. F. & Rubin, G. M. (1985) *Cell* **40**, 851–858.
10. Schinz, R. H., Lo, M.-V. C., Larrivee, D. C. & Pak, W. L. (1982) *J. Cell Biol.* **93**, 961–969.
11. Lindsley, D. L., Sandler, L., Baker, B. S., Carpenter, A. T. C., Denell, R. E., Hall, J. C., Jacobs, P. A., Miklos, G. L. G., Davis, B. K., Gethmann, R. C., Hardy, R. W., Hessler, A., Miller, S. M., Nozawa, H., Parry, D. M. & Gould-Somero, M. (1972) *Genetics* **71**, 157–184.
12. Lefevre, G., Jr. (1976) in *The Genetics and Biology of Drosophila*, eds. Ashburner, M. & Novitski, E. (Academic, New York), Vol. 1a, pp. 31–66.
13. Larrivee, D. C., Conrad, S. K., Stephenson, R. S. & Pak, W. L. (1981) *J. Gen. Physiol.* **78**, 521–545.
14. Scavarda, N. J., O'Tousa, J. & Pak, W. L. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4441–4445.
15. Fujita, S. & Hotta, Y. (1979) *Tanpakushitsu Kakusan Koso* **24**, 1336–1343.
16. Minke, B., Wu, C.-F. & Pak, W. L. (1975) *J. Comp. Physiol.* **98**, 345–355.
17. Lindsley, D. L. & Grell, E. H. (1968) *Genetic Variations of Drosophila melanogaster* (Carnegie Inst. Washington, Washington, DC).
18. O'Brien, S. J. & MacIntyre, R. J. (1978) in *The Genetics and Biology of Drosophila*, eds. Ashburner, M. & Wright, T. R. F. (Academic, New York), Vol. 2a, pp. 395–551.
19. Levy, L. S., Ganguly, R., Ganguly, N. & Manning, J. E. (1982) *Dev. Biol.* **94**, 451–464.
20. Rubin, G. M. & Spradling, A. C. (1982) *Science* **218**, 348–353.
21. Ruther, U. & Muller-Hill, B. (1983) *EMBO J.* **2**, 1791–1794.
22. Saibil, H. R. (1982) *J. Mol. Biol.* **158**, 435–456.
23. Blest, A. D., Stowe, S. & Eddey, W. (1982) *Cell Tissue Res.* **223**, 553–573.
24. Steinert, P. M., Steven, A. C. & Roop, D. R. (1985) *Cell* **42**, 411–419.
25. Johnson, E. C. & Pak, W. L. (1986) *J. Gen. Physiol.* **88**, 651–673.