Cell, Vol. 67, 255–263, October 18, 1991, Copyright © 1991 by Cell Press

The Cyclophilin Homolog ninaA Is Required in the Secretory Pathway

Nansi Jo Colley, Elizabeth K. Baker, Mark A. Stamnes, and Charles S. Zuker
Howard Hughes Medical Institute
and Departments of Biology and Neuroscience
University of California at San Diego
La Jolla, California 92093

Summary

In Drosophila, the major rhodopsin Rh1 is synthesized in endoplasmic reticulum (ER)-bound ribosomes of the R1–R6 photoreceptor cells and is then transported to the rhabdomeres where it functions in phototransduction. Mutations in the cyclophilin homolog ninaA lead to a 90% reduction in Rh1 opsin. Cyclophilins have been shown to be peptidyl-prolyl cis-trans isomerases and have been implicated in catalyzing protein folding. We now show that mutations in the *ninaA* gene severely inhibit opsin transport from the ER, leading to dramatic accumulations of ER cisternae in the photoreceptor cells. These results demonstrate that ninaA functions in the ER. Interestingly, ninaA and Rh1 also colocalize to secretory vesicles, suggesting that Rh1 may require ninaA as it travels through the distal compartments of the secretory pathway. These results are discussed in relation to the possible role of cyclophilins in protein folding and intracellular protein trafficking.

Introduction

The cyclophilins (CyPs) are a highly conserved family of proteins expressed ubiquitously in organisms from prokaryotes to mammals (reviewed by Hohman and Hultsch, 1990; Stamnes and Zuker, 1990; Schreiber, 1991). Many of these proteins display high binding affinity and stereospecificity for cyclosporin A (CsA) (Quesniaux et al., 1987). CsA is a small peptide of fungal origin with strong immunosuppressive properties (Borel, 1976; Borel et al., 1976) used to prevent graft rejection and in the treatment of autoimmune disorders (reviewed by Shevach, 1985; Kahan, 1989). CsA blocks an early event in the activation of helper T lymphocytes, primarily by inhibiting the transcription of T cell activation genes, such as interleukin-2, interleukin-4, and γ-interferon (Elliot et al., 1984; Kronke et al., 1984; Herold et al., 1986; Bickel et al., 1987; Drugge and Handschumacher, 1988; Granelli-Piperno, 1988; Emmel et al., 1989; Mattila et al., 1990; Randak et al., 1990). Although the mode of action of CsA is not known, whole-cell and biochemical studies support the notion that the immunosuppressive properties of CsA are mediated via its binding to CyP (reviewed by Sigal et al., 1990).

Sequence comparison among CyPs revealed the presence of a conserved central core domain (reviewed by Stamnes and Zuker, 1990), flanked in some cases by hydrophobic N-terminal extensions (Tropschug et al., 1988; Schneuwly et al., 1989; Shieh et al., 1989; Kawamukai et al., 1989; Iwai and Inagami, 1990; Liu and Walsh, 1990; Koser et al., 1990; Tropschug, 1990; Price et al., 1991; Caroni et al., 1991; Spik et al., 1991). The N-terminal extension may serve as a signal sequence and may be involved in the targeting of different CyPs to distinct subcellular compartments, thus raising the possibility that CyP heterogeneity may reflect functional diversity in this family of proteins.

A clue to the possible in vivo role of CyPs came from studies of their in vitro biochemical activities. CyPs are peptidyl-prolyl cis–trans isomerases (PPIases) that catalyze the cis–trans isomerization of a peptide bond between a proline residue and its amino-terminal neighbor. In vitro, PPIases accelerate the slow refolding phase of a number of denatured proteins, including collagen, ribonucleases, and immunoglobulins (Lang et al., 1987; Bächinger, 1987; Davis et al., 1989; Kiefhaber et al., 1990). Interestingly, CsA has been shown to be a potent inhibitor of the PPIase activity of CyPs (Fischer et al., 1989; Takahashi et al., 1989). Taken together, these findings provided a simple model for CyP action in T helper lymphocytes: CsA may inhibit the ability of CyP to catalyze the folding of key molecules involved in the T cell activation cascade. However, CsA analogs that bind CyP and inhibit its PPIase activity, but that do not immunosuppress, have been identified (Sigal et al., 1990, 1991). These results indicate that CyPs have other activities that are responsible for immunosuppression.

Recently, it has been proposed that CyPs may play a role in intracellular protein trafficking and in secretion (reviewed by Hohman and Hultsch, 1990; Schreiber, 1991; Caroni et al., 1991; Spik et al., 1991). Studies on the effect of CsA on the transcription factor NF-AT (nuclear factor of activated T cells) suggest that CsA may inhibit its cytoplasmic to nuclear translocation following T cell activation (Flanagan et al., 1991). In mast cells and cytotoxic T lymphocytes, CsA has been shown to inhibit calcium-dependent exocytosis (Trenn et al., 1989; Hultsch et al., 1990; reviewed by Hohman and Hultsch, 1990). These findings suggest CyPs have multiple roles in cellular metabolism (see Discussion).

Insights into the in vivo role of CyPs have come from studies of the *ninaA* gene in Drosophila. Mutations in the *ninaA* gene result in a major reduction in the levels of Rh1 rhodopsin in the R1–R6 photoreceptor cells (Larrivee et al., 1981). Previous studies showed that *ninaA* encodes a CyP homolog (Shieh et al., 1989; Schneuwly et al., 1989) that is essential for posttranslational processing of Rh1 (Zuker et al., 1988; Stamnes et al., 1991). ninaA is unique among CyPs in that it contains both a cleavable N-terminal signal sequence and a hydrophobic C-terminal transmembrane domain (Schneuwly et al., 1989; Shieh et al., 1989; Stamnes et al., 1991). It is a photoreceptor-specific integral membrane glycoprotein with the CyP-homologous domain located on the exoplasmic face of the membrane, making it ideally situated for a role in the Rh1 biosynthetic

pathway (Stamnes et al., 1991). We now show that ninaA is required for export of Rh1 opsin from the endoplasmic reticulum (ER) and that it is located throughout the secretory pathway. In addition, we show that *ninaA* mutant photoreceptor cells have dramatic accumulations of Rh1-laden ER cisternae. Interestingly, these mutant cells exhibit high specificity in their ability to process properly ninaA-dependent versus ninaA-independent rhodopsin substrates.

Results

Rhodopsin Is Not Translocated out of the ER in *ninaA* Mutant Photoreceptors

In wild-type Drosophila photoreceptor cells, Rh1 opsin is synthesized in the ER and transported via the secretory pathway to the rhabdomeres where it functions in visual transduction. Rhabdomeres are arrays of tightly packed microvilli containing the rhodopsin molecules and the machinery required for phototransduction. We have previously suggested that the ninaA protein is required for the proper folding, stability, or transport of Rh1 opsin during biosynthesis (Shieh et al., 1989; Stamnes et al., 1991). The improper processing of proteins targeted to the secretory pathway frequently leads to the retention of these proteins within the ER or other compartments along this pathway (Pfeffer and Rothman, 1987; Rose and Doms, 1988; Lodish, 1988; Hurtley and Helenius, 1989; Pelham, 1989; Doi et al., 1990). Thus, photoreceptors lacking ninaA may contain Rh1 that is not transported through the secretory pathway.

In wild-type flies, Rh1 opsin localizes predominantly to the rhabdomeres of the R1–R6 photoreceptors (Figure 1A). This is expected since fly rhodopsin has a very long half-life (W. S. Stark, personal communication), and at steady state the majority should localize to the rhabdomeres. In addition, small amounts of Rh1 are also found in phagosomes generated during the normal process of photoreceptor membrane turnover (see Figure 1A) (Blest, 1988; Stark et al., 1988). In sharp contrast, $ninaA^{269}$ null mutant flies have very little Rh1 opsin in their rhabdomeres. Most of the immunoreactivity is associated with the ER membranes (Figure 1B). These findings suggest that ninaA activity is required for the transport of Rh1 from the ER.

To examine this more closely, we used the glycosylation state of Rh1 to distinguish between rhabdomeric and ER opsin. Rh1 opsin has two potential sites for N-linked glycosylation (Asn-20 and Asn-193) (O'Tousa et al., 1985; Zuker et al., 1985). During biosynthesis, opsins in flies are transiently glycosylated in the ER (Huber et al., 1990); the mature molecules, present in the rhabdomeres, do not contain detectable levels of sugars (de Couet and Tanimura, 1987; Huber et al., 1990). We examined Rh1 opsin from control wild-type flies, from $ninaA^{269}$ mutants, and from a temperature-sensitive *ninaA* allele ($ninaA^{228}$), both at the restrictive (29°C) and permissive temperatures (18°C). Wild-type opsin and opsin from $ninaA^{228}$ mutants grown at 18°C consist mostly of the mature rhabdomeric form, while Rh1 opsin from either $ninaA^{269}$, or $ninaA^{228}$ grown at 29°C, contains a substantial amount of the high molecular weight glycosylated state (Figure 2, first panel). Treatment of $ninaA^{269}$ extracts with peptide N-glycosidase F or endoglycosidase H causes the electrophoretic mobility of the Rh1 opsin to increase such that it now comigrates with the mature form (Figure 2, second and third panels). Since endoglycosidase H only cleaves immature high mannosyl oligosaccharide chains (reviewed by Kornfeld and Kornfeld, 1985), the majority of Rh1 opsin must be in the ER.

ninaA Mutants Display Large Accumulations of ER

Examination of R1–R6 photoreceptors from *ninaA* mutants showed that these cells not only have reduced rhodopsin levels but also have an additional phenotype; the cells display large accumulations of rough ER membranes (Figures 3A and 3B). This massive ER buildup is composed of many long cisternae per ER stack often organized in concentric circles; in some cells these are so abundant, that they fill the cytoplasm. Serial sections through the eyes of $ninaA^{269}$ mutant flies confirmed that the ER accumulations are present in all of the R1–R6 photoreceptor cells, from apical to basal locations. In contrast, wild-type photoreceptors have a small amount of ER in the cytoplasm (see Figure 3D). The specificity of this phenotype was confirmed by examining $ninaA^{228}$ flies. ER accumulations are present at the restrictive temperature (Figure 3C), but not at the permissive temperature (Figure 3D).

Previous work from a number of laboratories has revealed that abnormal protein accumulations in the ER may cause overproliferation of both smooth and rough ER cisternae (Chin et al., 1982; Anderson et al., 1983; Wright et al., 1988; Pacifici and Iozzo, 1988). Thus, it is possible that Rh1 opsin itself is responsible for the buildup of ER membranes in *ninaA* mutants. To examine this possibility, we constructed a *ninaA*; *ninaE* double mutant. *ninaE* is the structural gene for the Rh1 opsin (O'Tousa et al., 1985; Zuker et al., 1985), so *ninaA*; *ninaE* flies lack ninaA and Rh1 opsin. Figure 3E shows that *ninaA*; *ninaE* double mutants do not display accumulations of ER membranes, demonstrating that it is not the lack of ninaA but rather the presence of Rh1 opsin in *ninaA* mutant photoreceptors that is responsible for the ER proliferation.

Recently, we showed that ninaA is required for the synthesis of the two closely related Drosophila opsins Rh1 and Rh2, but not for the two more distantly related Rh3 and Rh4 opsins (Stamnes et al., 1991). We have also examined the specificity of the ER phenotype by generating transgenic flies expressing Rh2, Rh3, or Rh4 opsins in the R1–R6 photoreceptors of *ninaA*; *ninaE* mutant hosts. Figure 3F shows that photoreceptors from transgenic animals in which we replaced the Rh1 opsin with Rh2 still display dramatic accumulations of ER membranes. However, R1–R6 photoreceptors expressing only the ninaA-independent Rh3 or Rh4 opsin displays normal ER (data not shown). Taken together, these results demonstrate that disruption of a specific interaction between ninaA and Rh1 (or Rh2) causes overproliferation of ER.

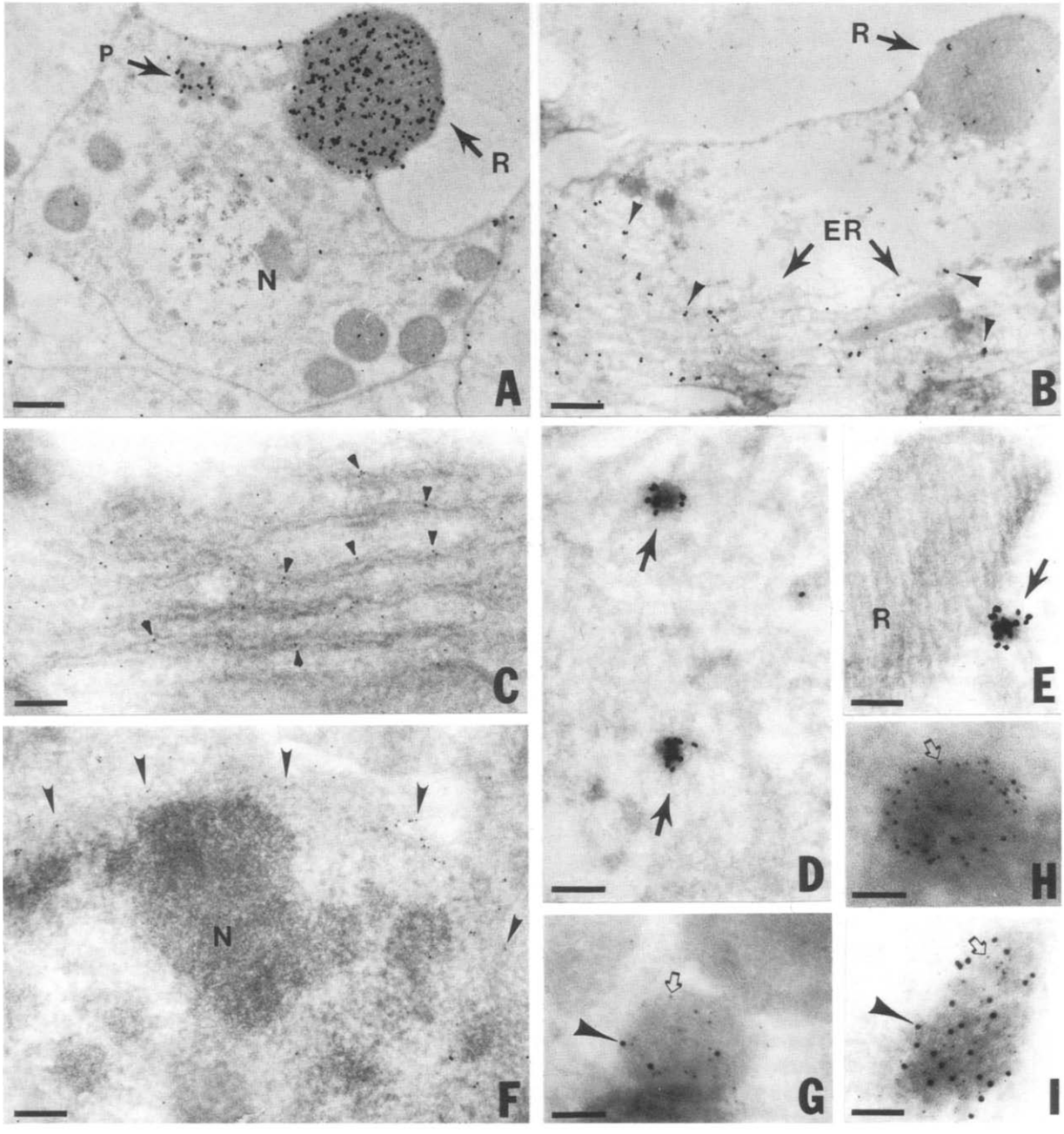

Figure 1. Immunolocalization of Rh1 and ninaA

Sections of photoreceptor cells embedded in Lowicryl 4KM were immunolabeled with 5 nm gold particles followed by silver enhancement (Janssen Silver Enhancement Kit) to demonstrate Rh1 opsin. (A) Photoreceptor cells from wild-type flies immunolabel for Rh1 opsin predominantly in the rhabdomeres (R). This section also shows a phagosome (P) containing Rh1 opsin; N, nucleus (bar, 0.5 μm). (B) ninaA^{269} photoreceptor cells display most of the Rh1 immunoreactivity in the ER (endoplasmic reticulum, small arrows), with very little in the rhabdomeres (bar, 0.45 μm). (C–F) Ultrathin cryosections of wild-type flies labeled with a rat anti-ninaA antibody (Stamnes et al., 1991) followed by 5 nm gold–conjugated goat anti-rat antibody. In (D) and (E) the gold particles were silver enhanced. Note the ninaA immunoreactivity in the following regions: (C) ER cisternae (arrowheads) (bar, 0.1 μm), (D and E) small vesicles located throughout the cytoplasm and at the base of the rhabdomeres (bars, 0.2 μm) (note that ninaA immunoreactivity is absent from the rhabdomeres [R]), and (F) nuclear envelope (bar, 0.2 μm). Cryosections of wild-type flies were also double immunolabeled with anti-ninaA (5 nm gold particles) and anti-Rh1 (15 nm) antibodies to colocalize ninaA and Rh1 opsin, respectively. (G) and (I) show vesicles that label for both ninaA and Rh1 opsin (arrowhead, Rh1; open arrow, ninaA protein). (H) shows a vesicle that labels only for ninaA. Bars, 0.1 μm (G), 0.05 μm (H), and 0.1 μm (I).

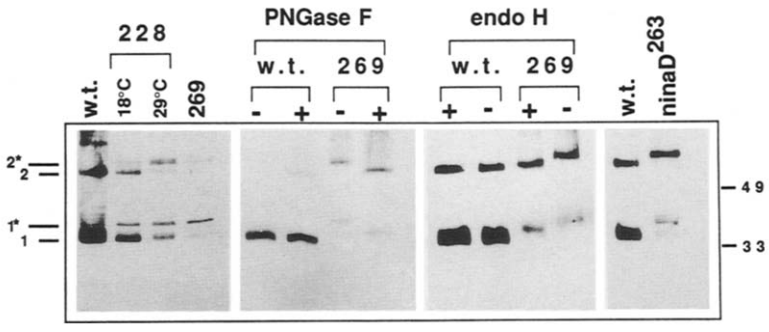

Figure 2. Rh1 Opsin Is Improperly Processed in *ninaA* and *ninaD* Mutants

Retinal extracts were prepared from wild-type controls, *ninaA*^{228}, *ninaA*^{269}, and *ninaD*^{263} mutants. Retinal proteins were separated by electrophoresis in 12% SDS–polyacrylamide gels, electroblotted to nitrocellulose, and labeled with a monoclonal anti–Rh1 opsin antibody as described in the Experimental Procedures. Drosophila Rh1 opsin can be found as mature, unglycosylated protein (1) or core glycosylated (1*). (2) and (2*) refer to the respective dimer forms of Rh1. Numbers to the right refer to molecular weight markers. Panel 1, wild-type Rh1 opsin and Rh1 from *ninaA*^{228} flies grown at 18°C are predominantly found in the lower molecular weight form, while Rh1 opsin from *ninaA* mutants (*ninaA*^{228} grown at 29°C and *ninaA*^{269}) is found in the immature higher molecular weight form (20 retinae per lane). Panel 2, retinal membrane preparations were incubated with 0.5 U of peptide-N-glycosidase F (+) as described in the Experimental Procedures and compared with undigested controls (−). Each wild-type control lane (w.t.) contains six retinae; each *ninaA*^{269} lane contains 60 retinae to compensate for the severe reduction of Rh1 levels in *ninaA* mutants (see first panel). Panel 3, retinal membranes were incubated with 0.0015 U of endoglycosidase H (+) as described in the Experimental Procedures and compared with undigested controls (−). Wild-type control lanes contain 10 retinae; each ninaA^{269} lane contains 100 retinae. Panel 4, a substantial amount of Rh1 opsin in *ninaD*^{263} mutants is also present in the higher molecular weight unprocessed form (60 retinae per lane), while Rh1 opsin from wild-type flies is exclusively composed of the mature form (two retinae per lane).

Given the dramatic ER phenotype of *ninaA* mutant photoreceptors, we wanted to assess whether ER processing of other proteins is also affected. To investigate this possibility we determined the expression pattern for another membrane protein, chaoptin, in *ninaA* mutant flies. Immunocytochemical analysis reveals that despite the huge ER accumulations in the photoreceptor cells, chaoptin is present at its normal location on the cell surface (data not shown) (see Van Vactor et al., 1988). In addition, the microvillar membranes of the rhabdomeres of *ninaA*^{269} flies do not display the disorganization that is characteristic of *chaoptic* mutant flies (Van Vactor et al., 1988). More specifically, we also examined whether the large ER accumulations are competent to process other opsins properly, like Rh3 and Rh4. We generated transgenic animals expressing the Rh2 and Rh4 opsins in the same photoreceptor cells. This was accomplished by directing expression of the *Rh2* and *Rh4* structural genes under the control of an R1–R6-specific promoter (Rh1 promoter). Photoreceptors of transgenic animals expressing both opsins still display large amounts of ER and produce defective Rh2 opsin when in a *ninaA* mutant background (data not shown). This is consistent with the requirement of ninaA for Rh2 biogenesis (Stamnes et al., 1991).

The presence of functional Rh4 rhodopsin in these transgenic flies was assayed electrophysiologically. R1–R6 photoreceptors undergo a prolonged depolarizing afterpotential (PDA), which persists after cessation of a light stimulus whenever a substantial amount of rhodopsin (R) is converted to the stable meta form (M) (approximately 20%–30% of wild-type levels must be photoconverted from R to M; Minke et al., 1975; Hillman et al., 1983). The PDA can be suppressed by photoconverting M back to R. Since the generation of a PDA requires photoconversion of a large amount of R to M, mutants with defective rhodopsin levels cannot undergo a PDA (Stephenson et al., 1983). We used the generation of a PDA as an indicator of the levels of functional Rh4 rhodopsin. Figure 4 shows that transgenic animals expressing Rh2 and Rh4 opsins in the R1–R6 cells of *ninaA* mutants still have sufficient levels of functional Rh4 rhodopsin, so as to generate a PDA, demonstrating that, in spite of the large accumulations of ER and retention of Rh2 opsin, a significant fraction of the Rh4 opsin is still properly processed.

If ER membrane buildup occurs when rhodopsin is improperly processed, then it follows that other mutations that affect opsin processing may also result in an accumulation of ER membranes. Thus, we examined the ultrastructure of photoreceptor cells from *ninaD* mutant flies. *ninaD* mutants are defective in retinal metabolism and fail to synthesize the retinal chromophore, which is normally covalently bound to the opsin apoprotein (Stephenson et al., 1983). Binding of chromophore to opsin is thought to take place in the ER (St. Jules et al., 1989). Ultrastructural analysis of photoreceptors from *ninaD*^{263} mutants shows a striking increase in ER content (Figure 3G). Moreover, most of the Rh1 opsin is in its high molecular weight unprocessed form, confirming that core glycosylated Rh1 opsin is not being transported out of the ER (Figure 2). Therefore, it appears that the retention of opsin in the ER cisternae and the accumulations of ER membranes are a general consequence of improper rhodopsin processing during biosynthesis. Thus, Rh1 opsin must interact with both ninaA and its chromophore to exit the ER.

The ninaA Protein Is Located in Several Compartments of the Secretory Pathway

To determine the exact subcellular location of the ninaA protein, we immunolabeled ultrathin cryosections of wild-type flies with an antibody directed to ninaA. As expected, ninaA immunolocalizes to the ER, often to cisternae associated with the outer nuclear envelope (Figures 1C and 1F). Surprisingly, ninaA is also found in small vesicles located throughout the cytoplasm (Figure 1D, 1E, and 1H).

If ninaA acts directly on Rh1 opsin, then we would expect to find both proteins located within the same compartments. Double immunolabeling experiments show that

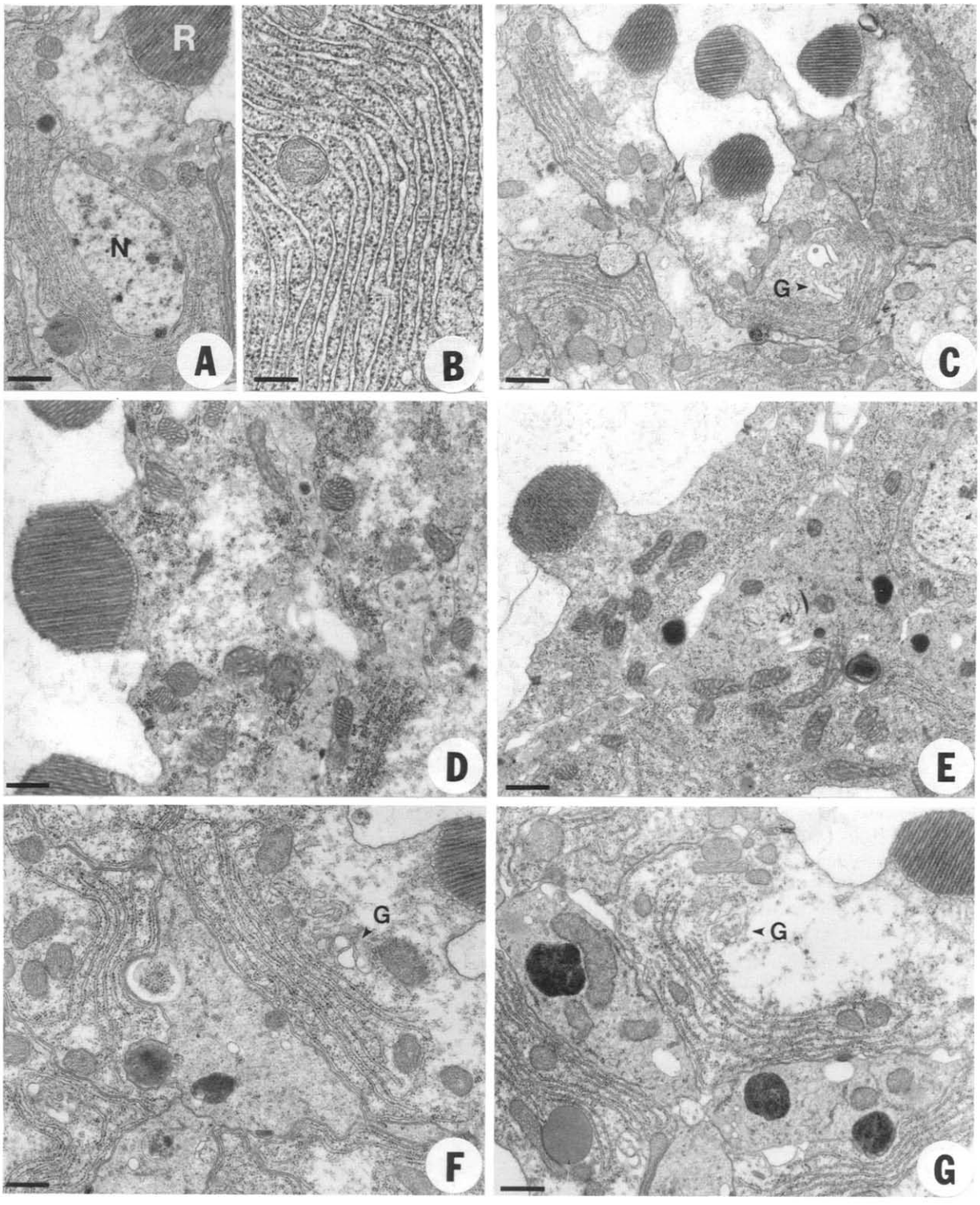

Figure 3. *ninaA* Mutants Overproduce ER

(A and B) Electron micrographs of ninaA^{269} photoreceptor cells displaying large accumulations of rough ER (bars, 0.45 μm and 0.3 μm, respectively). (C) Temperature-sensitive *ninaA*^{228} mutants grown at the restrictive temperature (29°C) overproduce ER (bar, 0.6 μm). (D) *ninaA*^{228} mutants grown at the permissive temperature (18°C) do not overproduce ER (bar, 0.4 μm). (E) The double mutant *ninaA*^{269}; *ninaE*^{117} does not display large accumulations of ER, demonstrating the Rh1 requirement for ER overproliferation (bar, 0.5 μm). (F) Transgenic flies expressing Rh2 opsin in the R1–R6 photoreceptor cells of *ninaA*^{269}; *ninaE*^{117} double mutants accumulate ER (bar, 0.5 μm). (G) ninaD^{263} photoreceptors also display large accumulations of ER (bar, 0.5 μm). R refers to rhabdomere, G refers to Golgi, and N refers to nucleus.

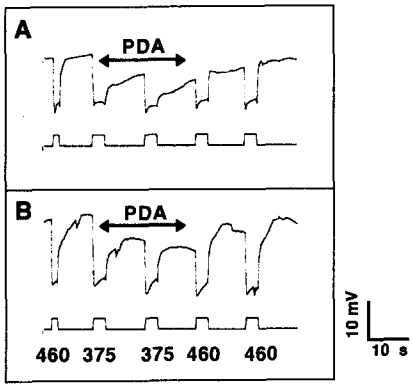

Figure 4. *ninaA* Mutants That Retain Rh2 Opsin in the ER Can Still Properly Process the Rh4 Rhodopsin

Shown are electroretinogram recordings from transgenic flies expressing the Rh2 and Rh4 opsins in the R1–R6 photoreceptor cells. The Rh1 promoter was used to direct the expression of the *Rh2* and *Rh4* structural genes. The host flies used for P element–mediated transformation were $ninaE^{117}$ and thus lacked all endogenous rhodopsin in their R1–R6 cells (O'Tousa et al., 1985). Responses to light stimuli representing the Rh4 absorption maxima (K. Kirschfeld, R. Feiler, R. Bjornson, M. Socolich, D. Smith, D. Mismer, G. M. Rubin, and C. S. Z., unpublished data) and of sufficient intensity to trigger a PDA are shown (see Experimental Procedures). PDAs are indicated by the double arrows. (A) represents recordings from control flies expressing Rh4 in the R1–R6 photoreceptor cells; note the robust PDA. (B) A recording from transgenic animals expressing the Rh4 and Rh2 opsins in the R1–R6 cells of *ninaA*; *ninaE* double mutants. These flies display large accumulations of ER due to the presence of improperly processed Rh2, yet note the PDA response of the Rh4 rhodopsin. The large depolarization to subsequent 375 nm light stimulus reflects activation of the small amount of Rh2 (λ max, 420 nm) and the ultraviolet-sensitizing pigment of the R1–R6 photoreceptors (Feller et al., 1988).

ninaA and Rh1 opsin colocalize in vesicles distributed throughout the cytoplasm (Figures 1G and 1I). These vesicles are presumably transporting Rh1 opsin from the ER and Golgi to the rhabdomeres. Interestingly, there are also examples of vesicles that contain predominantly one or the other molecule (Figure 1H). Since ninaA does not localize to the rhabdomeres (see Figure 1E), it is reasonable to assume that ninaA is sorted from Rh1 opsin at the base of the rhabdomere.

Discussion

The observation that mutations in the *ninaA* gene result in a block of Rh1 transport has important mechanistic implications. During biosynthesis, membrane proteins are thought to be inserted into the ER membrane in a largely unfolded state. Although relatively little is known about how they eventually attain their three-dimensional conformation, it is clear that protein export from the ER is often critically dependent on protein folding. Misfolded proteins tend to form insoluble aggregates that are presumably unable to enter transport vesicles, or they bind to resident ER proteins, such as heavy chain–binding protein (BiP, grp78), that prevent their export (reviewed by Rose and Doms, 1988; Lodish, 1988; Hurtley and Helenius, 1989; Pelham, 1989). The evidence presented in this paper points to ninaA as essential for the proper transport of Rh1 from the ER.

Mutations in the *ninaA* gene result in the accumulation of Rh1 in the ER and elaboration of massive amounts of ER membranes. Since membrane biogenesis requires the coordinated synthesis and assembly of all membrane components, this finding illustrates the dramatic changes in cellular membranes caused by the accumulation of a specific protein in the wrong location. Many examples of this phenomenon have been observed, including yeast and Chinese hamster ovary cells overexpressing 3-hydroxy-3-methylglutaryl coenzyme A reductase (Chin et al., 1982; Anderson et al., 1983; Wright et al., 1988), cultured chondroblasts accumulating procollagen (Pacifici and Iozzo, 1988), and ER accumulations in yeast *sec* mutants (Novick et al., 1980; Kaiser and Schekman, 1990). Proteins retained in the ER are often selectively degraded (Hurtley and Helenius, 1989); this is a possible explanation for the decreased levels of Rh1 in *ninaA* mutant flies. Given that ninaA immunolocalizes to the ER and shows homology to PPIase, all these results are consistent with ninaA playing a role in the folding of Rh1.

In general, proteins synthesized in the ER are sorted as permanent ER residents or are transported through the secretory pathway for further sorting. Export from the ER to the Golgi apparatus normally occurs by a nonselective bulk flow process, and specific sorting signals are required for the selective retention of soluble and membrane proteins in the ER (Munro and Pelham, 1986, 1987; Pfeffer and Rothman, 1987; Rothman, 1987; Lodish, 1988; Pelham, 1989, 1990). ninaA contains a C-terminal hydrophobic segment that has been shown to act as a membrane anchor domain and may serve as an ER retention signal.

An interesting difference between ninaA and most other ER proteins is that it is also found in distal compartments of the secretory pathway. It is possible that the colocalization of ninaA and Rh1 in secretory vesicles may reflect a functional requirement for ninaA in post-ER compartments. The high substrate specificity of ninaA for a distinct subset of rhodopsins, however, argues against a generalized role in the transport process per se. Interestingly, multiple lines of evidence point to CyPs as having additional activities besides PPIase activity. For instance, the identification of CsA analogs that inhibit the PPIase activity of CyP but that do not immunosuppress strongly argues that PPIase activity is not the CyP activity required in the activation of T helper lymphocytes (Sigal et al., 1990, 1991). One possibility is that CyPs are involved in intracellular transport events, including cytoplasmic–nuclear translocation (Flanagan et al., 1991) and calcium-dependent receptor-mediated secretion (reviewed by Hohman and Hultsch, 1990). Remarkably, recent data strongly support the notion that the CyP–CsA complex may have novel activities not found in uncomplexed CyP. For example, studies of Neurospora crassa and Saacharomyces cerevisiae mutants resistant to CsA have shown that these mutants either lack CyP or CyP has lost the ability to bind CsA, thus demonstrating that it is not the inhibition of CyP but rather the CyP–CsA complex that may be responsible for the toxic effects of CsA (Tropschug et al., 1989).

Therefore, unraveling the role of CyPs in cellular physiology will require not only a detailed study of the effects mediated by CsA, but more importantly, it will require a comprehensive analysis of the natural cellular function of CyPs. Dissecting the potentially complex functions of CyPs in catalysis of protein folding as well as in protein targeting and trafficking should provide valuable information into the biology of these processes. The work presented in this paper and the availability of Drosophila mutants defective in *ninaA* function are providing significant insights into the in vivo role of CyPs.

Experimental Procedures

Fly Stocks and Transgenic Animals

ninaA^{P269}, *ninaA*^{P228}, and *ninaD*^{P263} mutants were originally obtained from W. Pak. The *ninaE*^{I17} stock is a null allele of Rh1 containing an internal deletion in the *ninaE* gene (O'Tousa et al., 1985). The control stock used in this study was *w*^{1118}. *ninaA*^{P269}; *nina*^{EI17} double mutants and *w*^{1118}; *P[Rh1+2]*; *ninaA*^{P269}; *P[Rh1+4]*, *nina*^{EI17} animals were constructed using standard balancer stocks (Lindsley and Grell, 1968).

Transgenic flies expressing Rh2 or Rh4 opsin in the R1–R6 photoreceptor cells were constructed by generating transcriptional fusions between the Rh1 promoter and the structural gene for the minor opsins (see Zuker et al., 1988; Stamnes et al., 1991). The detailed microspectrophotometric and physiological analysis of the Rh1+4 lines will be presented elsewhere (K. Kirschfeld, R. Feiler, R. Bjornson, M. Socolich, D. Smith, D. Mismer, G. M. Rubin, and C. S. Z., unpublished data).

Electron Microscopy and Ultrastructural Analysis

Adult heads were fixed and processed according to the methods of Baumann and Walz (1989). The fixed tissue was dehydrated in serial changes of ethanol followed by propylene oxide and embedded in Spurr's medium (Polysciences, Inc.). Ultrathin sections were obtained on a Reichert Ultracut E ultramicrotome. Sections were stained with 2% uranyl acetate and lead citrate and viewed at 80 kV on a JOEL 1200EX electron microscope. For all genotypes described, at least five individual heads were sectioned, and 100 ommatidia were observed from each eye. During initial phases of the study, serial sections obtained from *w*^{1118} and *ninaA*^{P269} flies were observed to ensure that the phenotype was consistent from the apical to the basal regions of the eye. In all cases newly eclosed flies were used because in *ninaA* flies the number of ER cisternae appears to decrease with age.

Immunocytochemistry

Adult fly heads were fixed on ice in either 3% p-formaldehyde, 0.1% glutaraldehyde in 100 mM sodium phosphate buffer containing 2 mM calcium chloride (pH 7.2–7.4) or in 3% p-formaldehyde, 0.25% glutaraldehyde followed by sodium borohydride treatment (Eldred et al., 1983). The tissue was either infiltrated with 2.3 M sucrose and frozen in liquid nitrogen for cryoultramicrotomy (Tokuyasu, 1986, 1989) or dehydrated through a graded series of ethanols and embedded in Lowicryl 4KM according to the manufacturer's instructions (Electron Microscopic Sciences, Inc.). Polymerization in Lowicryl was carried out at −40°C under ultraviolet illumination. Ultrathin cryosections were obtained using a Reichert Ultracut E ultramicrotome equipped with an FC-4D cryo attachment. Sections were indirectly immunolabeled with a monoclonal antibody directed against Rh1 (a gift from D. Blest; de Couet and Tanimura, 1987), followed by a biotinylated goat anti-mouse immunoglobulin G (Jackson ImmunoResearch) and 5 nm gold–conjugated streptavidin (BioCell, Inc.), or 15 nm gold–conjugated goat anti-mouse antibody (Janssen Life Sciences Products). The affinity-purified rat anti-ninaA antibody (Stamnes et al., 1991) was followed by 5 nm gold–conjugated goat anti-rat antibody (Janssen Life Sciences Products). In some cases the gold particles were silver enhanced with AuroProbe EM according to the manufacturer's instructions (Janssen Life Sciences Products). The monoclonal antibody to chaoptin (24B10) was obtained from S. L. Zipursky (Van Vactor et al., 1988). After labeling, the sections were fixed in 2% glutaraldehyde, postfixed in 2% osmium tetroxide, and stained with 2% uranyl acetate. Cryosections were postembedded according to the methods described by Tokuyasu (1986, 1989). Sections were viewed at 80 kV on a JOEL 1200EX electron microscope.

Protein Gels and Western Blotting

Tissue homogenates were prepared as described by Stamnes et al. (1991). Retinal proteins were separated by electrophoresis in 12% SDS–polyacrylamide gels (Laemmli, 1970) and electroblotted onto nitrocellulose filters (Towbin et al., 1979). The nitrocellulose was incubated with a monoclonal antibody directed to the C-terminal region of Rh1 (de Couet and Tanimura, 1987), and the immunoreactive proteins were visualized using alkaline phosphatase–conjugated goat anti-mouse immunoglobulin G (Jackson ImmunoResearch). For glycosidase-treated samples, dissected retinas were homogenized in 20 mM Tris (pH 7.5), 0.1 mM phenylmethylsulfonyl fluoride, 0.5 μg/ml leupeptin, 0.5 μg/ml pepstatin and centrifuged at 100,000 × g for 30 min (Stamnes et al., 1991). Peptide-N-glycosidase (0.5 U; Boehringer Mannheim) or endoglycosidase H (0.0015 U; Boehringer Mannheim) treatments were carried out overnight at 37°C (Huber et al., 1990).

Electroretinograph Recordings

All recordings were carried on white-eyed flies. Glass or wick electrodes were filled with standard saline. Light stimulation was by means of a Xenon light beam (450 W Osram, Oriel Corp., Stratford, CT) passed through a high intensity grating monochromator (Oriel model 77264). The wavelengths required to trigger a PDA on transgenic flies expressing either the Rh2 or the Rh4 rhodopsin in the R1–R6 photoreceptors were determined experimentally by microspectrophotometry (Feiler et al., 1988; K. Kirschfeld, R. Feiler, R. Bjornson, M. Socolich, D. Smith, D. Mismer, G. M. Rubin, and C. S. Z., unpublished data). In transgenic animals coexpressing Rh2 and Rh4 opsins, the stimulating light was attenuated, and the band pass was decreased to reduce absorption overlap between these two rhodopsins.

Acknowledgments

We thank Michael McCaffery for his expert assistance during all aspects of the electron microscopical analysis and Drs. M. G. Farquhar and G. E. Palade and members of their laboratory for generous use of their facilities and insightful discussions. Ann Leslie and Lorraine Chuman produced the affinity-purified rat anti-ninaA antibodies. Drs. K. T. Tokuyasu, D. F. Ready, and J. E. O'Tousa provided helpful advice on the electron microscopy fixation procedures. We also thank Drs. M. G. Farquhar, H. F. Lodish, G. E. Palade, S. J. Singer, and members of the Zuker lab for advice on the manuscript. This work was supported by grants from the National Eye Institute to N. J. C. and C. S. Z. C. S. Z. acknowledges support from the Pew Foundation, the McKnight Foundation, the Alfred P. Sloan Foundation, and the Basil O'Connor program from the March of Dimes. C. S. Z. is an investigator of the Howard Hughes Medical Institute.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 USC Section 1734 solely to indicate this fact.

Received July 3, 1991; revised July 30, 1991.

References

Anderson, R. G. W., Orci, L., Brown, M. S., Garcia-Segura, L. M., and Goldstein, J. L. (1983). Ultrastructural analysis of crystalloid endoplasmic reticulum in Ut-1 cells and its disappearance in response to cholesterol. J. Cell Biol. *63*, 1–20.

Bächinger, H. P. (1987). The influence of peptidyl-prolyl cis-trans isomerase on the in vitro folding of type III collagen. J. Biol. Chem. *262*, 17144–17148.

Baumann, O., and Walz, B. (1989). Topography of Ca^{2+}-sequestering endoplasmic reticulum in photoreceptors and pigmented glial cells in the compound eye of the honeybee drone. Cell Tissue Res. *255*, 511–522.

Bickel, M., Tsuda, H., Amstad, P., Evequoz, V., Mergenhagen, S. E., Wahl, S. M., and Pluznik, D. H. (1987). Differential regulation of

colony-stimulating factors and interleukin 2 production by cyclosporin A. Proc. Natl. Acad. Sci. USA *84*, 3274–3277.

Blest, A. D. (1988). The turnover of phototransductive membrane in compound eyes and ocelli. Adv. Insect Physiol. *20*, 1–53.

Borel, J. F. (1976). Comparative study of in vitro and in vivo drug effects on cell-mediated cytotoxicity. Immunology *31*, 631–641.

Borel, J. F., Feurer, C., Gubler, H. U., and Stahelin, H. (1976). Biological effects of cyclosporin A: a new antilymphocytic agent. Agents Actions *6*, 468–475.

Caroni, P., Rothenfluh, A., McGlynn, E., and Schneider, C. (1991). S-cyclophilin. J. Biol. Chem. *266*, 10739–10742.

Chin, D. J., Luskey, K. L., Anderson, R. G. W., Faust, J. R., Goldstein, J. L., and Brown, M. S. (1982). Appearance of crystalloid endoplasmic reticulum in compactin-resistant Chinese hamster cells with a 500-fold increase in 3-hydroxy- 3-methylglutaryl-coenzyme A reductase. Proc. Natl. Acad. Sci. USA *79*, 1185–1189.

Davis, J. M., Boswell, B. A., and Bächinger, H. P. (1989). Thermal stability and folding of type IV collagen and effect of peptidyl-prolyl cis-trans-isomerase on the folding of the triple helix. J. Biol. Chem. *264*, 8956–8962.

de Couet, H. G., and Tanimura, T. (1987). Monoclonal antibodies provide evidence that rhodopsin in the outer rhabdomeres of *Drosophila melanogaster* is not glycosylated. Eur. J. Cell Biol. *44*, 50–56.

Doi, T., Molday, R. S., and Khorana, H. G. (1990). Role of the intradiscal domain in rhodopsin assembly and function. Proc. Natl. Acad. Sci. USA *87*, 4991–4995.

Drugge, R. J., and Handschumacher, R. E. (1988). Cyclosporine—mechanism of action. Transplant. Proc. *20*, 301–309.

Eldred, W. D., Zucker, C., Karten, H. J., and Yazulla, S. (1983). Comparison of fixation and penetration enhancement techniques for use in ultrastructural immunocytochemistry. J. Histochem. Cytochem. *31*, 285–292.

Elliot, J. F., Lin, Y., Mizel, S. B., Bleackley, R. C., Harnish, D. G., and Paetkau, V. (1984). Induction of interleukin-2 messenger RNA inhibited by cyclosporin A. Science *226*, 1439–1441.

Emmel, E. A., Verweij, C. L., Durand, D. B., Higgins, K. M., Lacy, E., and Crabtree, G. R. (1989). Cyclosporin A specifically inhibits function of nuclear proteins involved in T cell activation. Science *246*, 1617–1620.

Feiler, R., Harris, W. A., Kirschfeld, K., Wehrhahn, C., and Zuker, C. S. (1988). Targeted misexpression of a *Drosophila* opsin gene leads to altered visual function. Nature *333*, 737–741.

Fischer, G., Wittmann-Liebold, B., Lang, K., Kiefhaber, T., and Schmid, F. X. (1989). Cyclophilin and peptidyl-prolyl cis-trans isomerase are probably identical proteins. Nature *337*, 476–478.

Flanagan, W. M., Corthésy, B., Bram, R. J., and Crabtree, G. R. (1991). Nuclear association of a T cell transcription factor blocked by FK-506 and cyclosporin A. Nature *352*, 803–807.

Granelli-Piperno, A. (1988). In situ hybridization for interleukin-2 and interleukin-2 receptor mRNA in T cells activated in the presence or absence of cyclosporin A. J. Exp. Med. *168*, 1649–1658.

Herold, K. C., Lancki, D. W., Moldwin, R. L., and Fitch, F. W. (1986). Immunosuppressive effects of cyclosporin A on cloned T cells. J. Immunol. *136*, 1315–1321.

Hillman, P., Hochstein, S., and Minke, B. (1983). Transduction in invertebrate photoreceptors: role of pigment bi-stability. Physiol. Rev. *63*, 668–772.

Hohman, R. J., and Hultsch, T. (1990). Cyclosporin A: new insights for cell biologists and biochemists. New Biologist *2*, 663–672.

Huber, A., Smith, D. P., Zuker, C. S., and Paulsen, R. (1990). Opsin of Calliphora peripheral photoreceptors R1–6. J. Biol. Chem. *265*, 17906–17910.

Hultsch, T., Rodriguez, J. L., Kaliner, M. A., and Hohman, R. J. (1990). Cyclosporin A inhibits degranulation of rat basophilic leukemia cells and human basophils. J. Immunol. *144*, 2659–2664.

Hurtley, S. M., and Helenius, A. (1989). Protein oligomerization in the endoplasmic reticulum. Annu. Rev. Cell Biol. *5*, 277–307.

Iwai, N., and Inagami, T. (1990). Molecular cloning of a complementary DNA to rat cyclophilin-like protein mRNA. Kidney Int. *37*, 1460–1465.

Kahan, B. D. (1989). Drug therapy—cyclosporine. N. Engl. J. Med. *321*, 1725–1738.

Kaiser, C. A., and Schekman, R. (1990). Distinct sets of *SEC* genes govern transport vesicle formation and fusion early in the secretory pathway. Cell *61*, 723–733.

Kawamukai, M., Matsuda, H., Fujii, W., Utsumi, R., and Komano, T. (1989). Nucleotide sequences of *fic* and *fic-1* genes involved in cell filamentation induced by cyclic AMP in *Escherichia coli*. J. Bacteriol. *171*, 4525–4529.

Kiefhaber, T., Quaas, R., Hahn, U., and Schmid, F. X. (1990). Folding of ribonuclease T1. 1. Existence of multiple unfolded states created by proline isomerization. Biochemistry *29*, 3053–3061.

Kornfeld, R., and Kornfeld, S. (1985). Assembly of asparagine linked oligosaccharides. Annu. Rev. Biochem. *54*, 631–664.

Koser, P. L., Sylvester, D., Livi, G. P., and Bergsma, D. J. (1990). A second cyclophilin-related gene in *Saccharomyces cerevisiae*. Nucl. Acids Res. *18*, 1643.

Kronke, M., Leonard, W. J., Depper, J. M., Arya, S. K., Wong-Staal, F., Gallo, R. C., Waldman, T. A., and Greene, W. C. (1984). Cyclosporin A inhibits T-cell growth factor gene expression at the level of mRNA transcription. Proc. Natl. Acad. Sci. USA *81*, 5214–5218.

Laemmli, U. K. (1970). Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature *227*, 680–685.

Lang, K., Schmid, F. X., and Fischer, G. (1987). Catalysis of protein folding by prolyl isomerase. Nature *329*, 268–270.

Larrivee, D. C., Conrad, S., Stephenson, R. S., and Pak, W. L. (1981). Mutation that selectively affects rhodopsin concentration in the peripheral photoreceptors of *Drosophila melanogaster*. J. Gen. Physiol. *78*, 521–545.

Lindsley, D. L., and Grell, E. H. (1968). Genetic variations of *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. 627.

Liu, J., and Walsh, C. T. (1990). Peptidyl-prolyl *cis- trans*-isomerase from *Escherichia coli*: a periplasmic homolog of cyclophilin that is not inhibited by cyclosporin A. Proc. Natl. Acad. Sci. USA *87*, 4028–4032.

Lodish, H. F. (1988). Transport of secretory and membrane glycoproteins from the rough endoplasmic reticulum to the Golgi. J. Biol. Chem. *263*, 2107–2110.

Mattila, P. S., Ullman, K. S., Fiering, S., Emmel, E. A., McCutcheon, M., Crabtree, G. R., and Herzenberg, L. A. (1990). The actions of cyclosporin A and FK506 suggest a novel step in the activation of T lymphocytes. EMBO J. *9*, 4425–4433.

Minke, B., Wu, C. F., and Pak, W. L. (1975). Isolation of light-induced response of the central retinula cells from the electroretinogram of *Drosophila*. J. Comp. Physiol. *98*, 345–355.

Munro, S., and Pelham, H. R. B. (1986). An hsp70-like protein in the ER: identity with the 78 kd glucose-regulated protein and immunoglobulin heavy chain binding protein. Cell *46*, 291–300.

Munro, S., and Pelham, H. R. B. (1987). A C-terminal signal prevents secretion of luminal ER proteins. Cell *48*, 899–907.

Novick, P., Field, C., and Schekman, R. (1980). Identification of 23 complementation groups required for post-translational events in the yeast secretory pathway. Cell *21*, 205–215.

O'Tousa, J. E., Baehr, W., Martin, R. L., Hirsh, J., Pak, W. L., and Applebury, M. L. (1985). The Drosophila *ninaE* gene encodes an opsin. Cell *40*, 839–850.

Pacifici, M., and Iozzo, R. V. (1988). Remodeling of the rough endoplasmic reticulum during stimulation of procollagen secretion by ascorbic acid in cultured chrondrocytes. J. Biol. Chem. *263*, 2483–2492.

Pelham, H. R. B. (1989). Control of protein exit from the endoplasmic reticulum. Annu. Rev. Biol. *5*, 1–23.

Pelham, H. R. B. (1990). The retention signal for soluble proteins of the endoplasmic reticulum. Trends Biochem. Sci. *15*, 483–486.

Pfeffer, S. R., and Rothman, J. E. (1987). Biosynthetic protein transport and sorting by the endoplasmic reticulum and Golgi. Annu. Rev. Biochem. *56*, 829–852.

Price, E. R., Zydowsky, L. D., Jin, M., Baker, C. H., McKeon, F. D., and Walsh, C. T. (1991). Human cyclophilin B: a second cyclophilin gene encodes a peptidyl-prolyl isomerase with a signal sequence. Proc. Natl. Acad. Sci. USA *88*, 1903–1907.

Quesniaux, V. F. J., Schreier, M. H., Wenger, R. M., Hiestand, P. C., Harding, M. W., and Van Regenmortel, M. H. V. (1987). Cyclophilin binds to the region of cyclosporine involved in its immunosuppressive activity. Eur. J. Immunol. *17*, 1359–1365.

Randak, C., Brabletz, T., Hergenrother, M., Sobotta, I., and Serfling, E. (1990). Cyclosporin A suppresses the expression of the interleukin 2 gene by inhibiting the binding of lymphocyte-specific factors to the IL-2 enhancer. EMBO J. *9*, 2529–2536.

Rose, J. K., and Doms, R. W. (1988). Regulation of protein export from the endoplasmic reticulum. Annu. Rev. Cell Biol. *4*, 257–288.

Rothman, J. E. (1987). Protein sorting by selective retention in the endoplasmic reticulum and Golgi stack. Cell *50*, 521–522.

Schneuwly, S., Shortridge, R. D., Larrivee, D. C., Ono, T., Ozaki, M., and Pak, W. L. (1989). *Drosophila ninaA* gene encodes an eye-specific cyclophilin (cyclosporin A binding protein). Proc. Natl. Acad. Sci. USA *86*, 5390–5394.

Schreiber, S. L. (1991). Chemistry and biology of the immunophilins and their immunosuppressive ligands. Science *251*, 283–287.

Shevach, E. M. (1985). The effects of cyclosporin A on the immune system. Annu. Rev. Immunol. *3*, 397–423.

Shieh, B.-H., Stamnes, M. A., Seavello, S., Harris, G. L., and Zuker, C. S. (1989). The *ninaA* gene required for visual transduction in *Drosophila* encodes a homologue of cyclosporin A-binding protein. Nature *338*, 67–70.

Sigal, N. H., Siekierka, J. J., and Dumont, F. J. (1990). Observations on the mechanism of action of FK-506, a pharmacological probe of lymphocyte signal transduction. Biochem. Pharmacol. *40*, 2201–2208.

Sigal, N. H., Dumont, F., Durette, P., Siekierka, J. J., Peterson, L., Rich, D. H., Dunlap, B. E., Staruch, M. J., Melino, M. R., Koprak, S. L., Williams, D., Witzel, B., and Pisano, J. M. (1991). Is cyclophilin involved in the immunosuppressive and nephrotoxic mechanism of action of cyclosporin A? J. Exp. Med. *173*, 619–628.

Spik, G., Haendler, B., Delmas, O., Mariller, C., Chamoux, M., Maes, P., Tarter, A., Montreuil, J., Stedman, K., Kocher, H. P., Keller, R., Hiestand, P. C., and Movva, N. R. (1991). A novel secreted cyclophilin-like protein (SCYLP). J. Biol. Chem. *266*, 10735–10738.

Stamnes, M. A., and Zuker, C. S. (1990). Peptidyl-prolyl *cis-trans* isomerases, cyclophilin, FK506-binding protein, and ninaA: four of a kind. Curr. Opin. Cell Biol. *2*, 1104–1107.

Stamnes, M. A., Shieh, B.-H., Chuman, L., Harris, G. L., and Zuker, C. S. (1991). The cyclophilin homolog ninaA is a tissue-specific integral membrane protein required for the proper synthesis of a subset of Drosophila rhodopsins. Cell *65*, 219–227.

Stark, W. S., Sapp, R., and Schilly, D. (1988). Rhabdomere turnover and rhodopsin cycle: maintenance of retinula cells in *Drosophila melanogaster*. J. Neurocytol. *17*, 499–509.

Stephenson, R. S., O'Tousa, J., Scavarda, N. J., Randall, L. L., and Pak, W. L. (1983). *Drosophila* mutants with reduced rhodopsin content. In Biology of Photoreceptors, D. Cosens and D. Vince-Price, eds. (Cambridge, England: Cambridge University Press), pp. 477–501.

St. Jules, R. S., Wallingford, J. C., Smith, S. B., and O'Brien, P. J. (1989). Addition of the chromophore to rat rhodopsin is an early post-translational event. Exp. Eye Res. *48*, 653–665.

Takahashi, N., Hayano, T., and Suzuki, M. (1989). Peptidyl-prolyl cis-trans isomerase is the cyclosporin A-binding protein cyclophilin. Nature *337*, 473–475.

Tokuyasu, K. T. (1986). Application of cryoultramicrotomy to immunocytochemistry. J. Microsc. *143*, 139–149.

Tokuyasu, K. T. (1989). Use of poly(vinylpyrrolidone) and poly(vinyl alcohol) for cryoultramicrotomy. Histochem. J. *21*, 163–171.

Towbin, H., Staehelin, T., and Gordon, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets. Procedure and some applications. Proc. Natl. Acad. Sci. USA *76*, 4350–4354.

Trenn, G., Taffs, R., Hohman, R., Kincaid, R., Shevach, E. M., and Sitkovsky, M. (1989). Biochemical characterization of the inhibitory effect of CsA on cytolytic T lymphocyte effector functions. J. Immunol. *142*, 3796–3802.

Tropschug, M. (1990). Nucleotide sequence of the gene coding for cyclophilin/peptidyl-prolyl *cis-trans* isomerase of *Neurospora crassa*. Nucl. Acids Res. *18*, 190.

Tropschug, M., Nicholson, D. W., Hartl, F., Küöhler, H., Pfanner, N., Wachter, E., and Neupert, W. (1988). Cyclosporin A-binding protein (cyclophilin) of *Neurospora crassa*. J. Biol. Chem. *263*, 14433–14440.

Tropschug, M., Barthelmess, I. B., and Neupert, W. (1989). Sensitivity to cyclosporin A is mediated by cyclophilin in *Neurospora crassa* and *Saccharomyces cerevisiae*. Nature *342*, 953–955.

Van Vactor, D., Jr., Krantz, D. E., Reinke, R., and Zipursky, S. L. (1988). Analysis of mutants in chaoptin, a photoreceptor cell–specific glycoprotein in Drosophila, reveals its role in cellular morphogenesis. Cell *52*, 281–290.

Wright, R., Basson, M., D'Ari, L., and Rine, J. (1988). Increased amounts of HMG-CoA reductase induce "Karmellae" a proliferation of stacked membrane pairs surrounding the yeast nucleus. J. Cell Biol. *107*, 101–114.

Zuker, C. S., Cowman, A. F., and Rubin, G. M. (1985). Isolation and structure of a rhodopsin gene from D. melanogaster. Cell *40*, 851–858.

Zuker, C. S., Mismer, D., Hardy, R., and Rubin, G. M. (1988). Ectopic expression of a minor Drosophila opsin in the major photoreceptor cell class: distinguishing the role of primary receptor and cellular context. Cell *53*, 475–482.