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Potential Cellular Functions of *N*-Ethylmaleimide Sensitive Factor in the Photoreceptor

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101.1 Introduction

Vesicle traffic and fusion are essential, not only for cellular homeostasis but also for neuronal signal transmission across the synaptic junction of nerves, cell growth, and membrane repair. The basic fusion process is mediated by vesicle (v)-soluble *N*-ethylmaleimide sensitive factor attachment proteins receptors (SNAP receptors; SNAREs) on the secretory vesicle with their cognate target (t)-SNAREs on the target membrane (Sollner et al. 1993), which assemble in trans-configuration into four-helix bundle complexes, bringing the membranes into close proximity (Jahn and Scheller 2006; Rizo and Rosenmund 2008; Martens and McMahon 2008), which then directly or indirectly leads to fusion. After membrane fusion, all SNAREs constituting one complex are anchored in a relaxed *cis*-configuration to one membrane. Disassembly of these SNAREs complexes for subsequent vesicle transport and recycling is achieved by the concerted action of α-SNAP and NSF.

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N-ethylmaleimide sensitive factor (NSF) is a homo-hexameric member of the ATPase-associated family with various cellular activities protein (AAA) family, required for intracellular membrane fusion. NSF functions as a SNAP receptor (SNARE) chaperone that binds through soluble NSF attachment proteins (SNAPs) to SNARE complexes and utilizes the energy of ATP hydrolysis to dissociate SNARE complexes after membrane fusion, thus facilitating SNAREs recycling.

Each NSF protomer contains an N-terminal domain (NSF-N) and two AAA-domains: a catalytic NSF-D1 and a structural NSF-D2 (Fig. 101.1). The amino acid residues 1–205 in the NSF-N domain are required for SNAP–SNARE binding; residues 206–477 in the NSF-D1 domain are responsible for the majority of the ATP hydrolysis, while the carboxy-terminal residues 478–744 in the NSF-D2 domain are required for hexamerization (Tagaya et al. 1993; Nagiec et al. 1995). Within the N-terminal subdomain of both NSF-D1 and NSF-D2, there is a highly conserved region called Second Region of Homology (SRH), which is highly conserved in AAA proteins (Hanson and Whiteheart 2005). By using detailed mutagenesis analysis, Zhao and collaborators (Zhao et al. 2010) showed that a positively charged surface on NSF-N, bounded by R67 and K105, and the conserved central pore motifs in NSF-D1 (Y296 and G298) are involved in SNAP–SNARE binding but not basal ATP hydrolysis. Sensor 1 is at the N-terminus of the SRH and is important for basal ATPase activity and nucleotide binding. At its carboxy-terminus are two arginine residues, termed Arginine Fingers, which are critical for ATP hydrolysis by the NSF hexamer. Sensor 2 comes from C-terminal helical subdomain and plays a role in ATP- and SNAP-dependent SNARE complex binding and disassembly.

NSF binds to SNARE complexes via its adaptor protein, a-SNAP, only in the presence of ATP (Nagiec et al. 1995). The intrinsic ATPase activity of NSF is low (Tagaya et al. 1993). NSF binding to immobilized a-SNAP stimulates the ATPase activity (Morgan et al. 1994), and maximal stimulation of ATPase activity is achieved when both α-SNAP and SNARE complexes are included (Matveeva and Whiteheart 1998).

101.2 NSF in Photoreceptor Synaptic Regulation

In the G-protein-coupled receptor (GPCR) phototransduction cascade, visual Arrestin1 (Arr1) binds to and deactivates phosphorylated, light-activated opsins, a process that is critical for effective recovery and normal vision. We discovered a novel synaptic protein–protein interaction between Arr1 and NSF that is enhanced in a dark environment when photoreceptors are depolarized and the rate of exocytosis is elevated compared to a light environment (Huang et al. 2010; Huang 2010) (Fig. 101.2a, b). In Figs. 101.2 and 101.3, representative images of sections from either dark- or light-adapted retinas highlight differences in immunohistochemical retinal dual localization in mouse of Arr1 and NSF, respectively (Figs. 101.2a–d and 101.3a–d). We also provided convincing in vitro evidence supporting the interaction of Arr1 with NSF to modulate its ATPase activity and to drive disassembly of the SNARE complex. Furthermore, we observed with in vivo studies using FM-143 imaging that synaptic vesicle recycling in photoreceptors is dramatically decreased with depolarization and photopic electroretinogram (ERG) b-wave analysis in *Arr1*$^{-/-}$ compared to control mice is defective in light adaptation (Brown et al. 2010). Not only

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does Arr1 bind to the junction of NSF N-terminal and the first ATPase domains in an ATP-dependent manner, but Arr1 also enhances both NSF ATPase and NSF disassembly activities. In mouse retinas with no Arr1 expression, the expression levels of NSF and other synapse-enriched genes are markedly reduced and lead to a substantial decrease in the exocytosis rate. These cumulative findings demonstrate that normal photoreceptor synaptic function involves the ability of Arr1 to regulate and to enhance the dark-associated activity of NSF in the photoreceptor synapse.

101.3 NSF in Photoreceptor Membrane Protein Trafficking

While the major function of NSF is involved in vesicle transport and recycling, it also interacts with other proteins such as the AMPA receptor subunit (Osten et al. 1998), β-arrestin1 (McDonald et al. 1999), GluR2 (Nishimune et al. 1998), and β2-AR (Cong et al. 2001) and is thought to affect their trafficking pattern. More evidence suggests that NSF may be regulated by transient posttranslational modifications such as phosphorylation and nitrosylation. These modifications are ideal mechanisms for reversible regulation of membrane trafficking.

Recently, Molday and his collaborators observed that NSF specifically interacts with RP2 in the retina (Holopainen et al. 2010), which is a ubiquitously expressed protein encoded by a gene associated with X-linked retinitis pigmentosa (Breuer et al. 2002; Miano et al. 2001; Schwahn et al. 1998; Sharon et al. 2003). RP2 is known to bind to the GTP-bound form of ADP ribosylation factor like 3 (Arl3), a member of the Arl subfamily of Ras-related GTP-binding proteins (Bartolini et al. 2002; Kuhnel et al. 2006; Veltel et al. 2008) and acts as an efficient GTPase-activating protein (GAP) for Arl3 (Veltel et al. 2008). NSF and RP2 was shown to colocalize in the ciliary region, inner segment, outer nuclear layer, and synaptic region in the photoreceptor. Furthermore, RP2 binds to the N-terminal domain of NSF and this binding is abolished for the E138G and I137 mutation of RP2 known to cause X-linked retinitis pigmentosa. These data may indicate RP2 binding to NSF, and Arl3 may play a crucial role in the vesicle trafficking of proteins to the photoreceptor ciliary region as well as in the photoreceptor synapse.

101.4 Summary

Recent work has established potential new functional roles for NSF in the photoreceptor. First, the interaction of Arr1 and NSF is ATP-dependent, and the N-terminal domain of Arr1 interacts with the N and D1 junctional domains of NSF. The Arr1–NSF interactions are greater in the photoreceptor synaptic terminal in the dark. Furthermore, Arr1 enhances the NSF ATPase activity and increases the NSF disassembly activities, which are critical for NSF functions in sustaining a higher rate of exocytosis in the photoreceptor synapses and the compensatory endocytosis to retrieve vesicle membrane and vesicle proteins for vesicle recycling. These data demonstrate the Arr1 and NSF interaction are necessary for both maintenance and modulation of normal photoreceptor synaptic regulation. Second, NSF colocalizes and specifically binds to RP2, especially in the ciliary and synaptic region of the photoreceptor, and NSF–RP2 interaction may play an important role in membrane protein trafficking in the photoreceptor.

Inherited retinal degeneration affects about 1 in 2,000–3,000 individuals in the world and is the leading cause of visual loss in young people and accounts for a large proportion of blindness in adult life. These studies accelerate our ability to gain insight into the diverse roles of the NSF in the photoreceptor cells and enable us to understand more precisely the molecular mechanisms underlying night blindness associated with clinically diagnosed Oguchi disease or other forms of retinitis pigmentosa.

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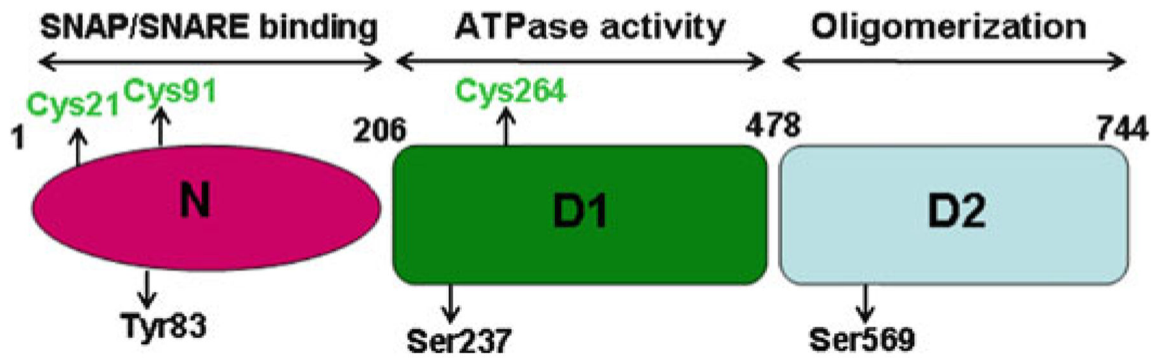

Fig. 101.1.

Structure of NSF domains. Each NSF monomer is comprised of three domains: an N-terminal domain (amino acid 1–205) that is responsible for the interaction with a-SNAP and SNAREs; and two homologous ATP-binding domains, D1 (amino acids 206–477), in which the hydrolytic activity is associated with NSF-driven SNARE complex disassembly, and D2 (amino acids 478–744), which is responsible for hexamer formation. Critical residues involved in posttranslational processing include Cys21, 91 and 264, Tyr83, Ser237, and Ser569

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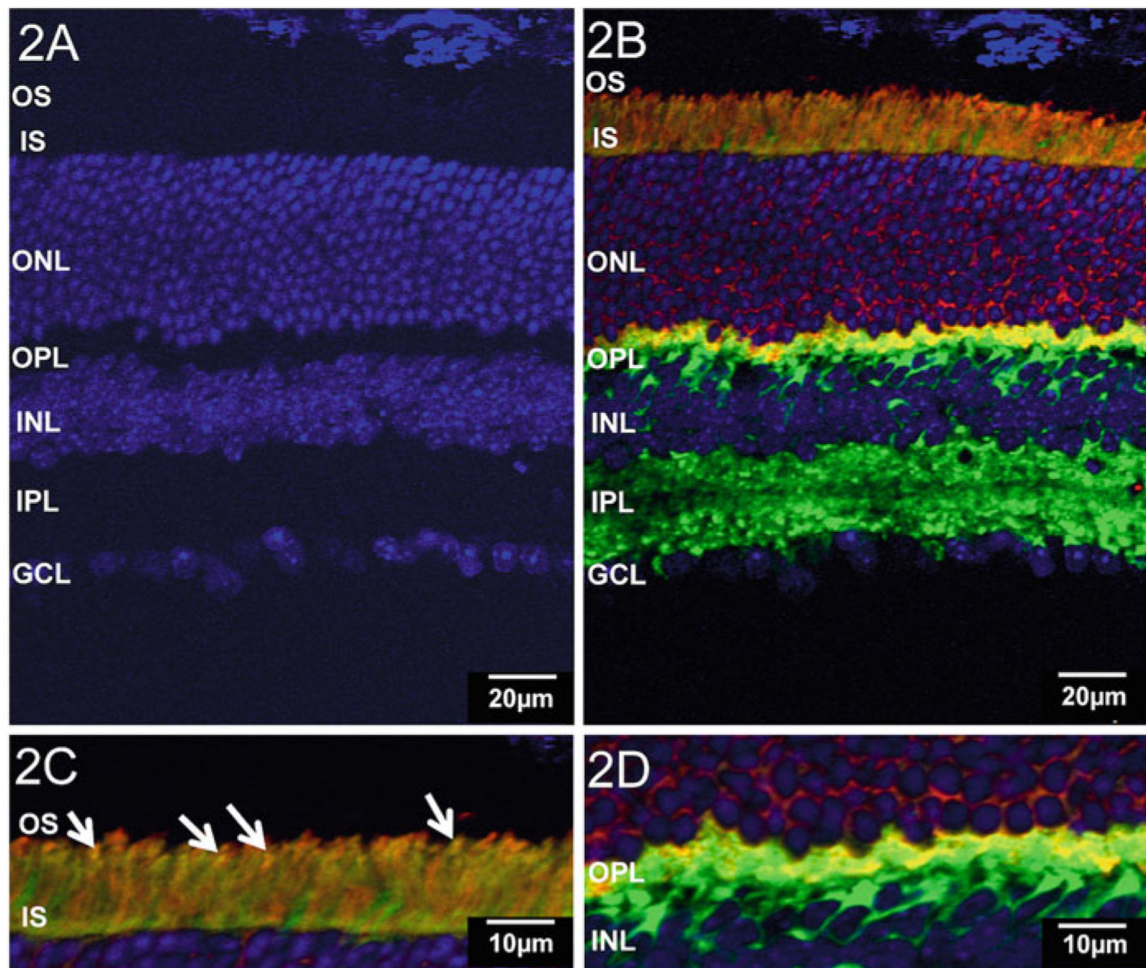

Fig. 101.2.

NSF and Arr1 localization in dark-adapted retinas. Immunohistochemical fluorescent labeling of retinal sections with dual localization of NSF and Arr1 staining in the WT mice dark-adapted (DA) overnight and killed in the dark. Adult WT mouse retina frozen sections were triple labeled fluorescently with the antimouse Arr1 MAb D9F2 (*red*, 1:20,000), antirabbit NSF PAb (*green*, 1:2,500, Abcam), and secondary antibodies conjugated to Alexa Fluor 488 or 568, respectively (1:500, invitrogen), and DAPI for the nuclei (*blue*, (**a**, **b**)). NSF immunoreactive staining pattern of NSF is mainly in the OPL and IPL in dark-adapted retinas (**c**, **d**). Arr1 immunoreactive pattern is predominantly in the inner segment, perinuclear area and a fraction in the photoreceptor terminal in DA retinas ((**c**), higher magnification; *large arrows*). *OS* outer segment; *IS* inner segments; *ONL* outer nuclear layer; *OPL* outer plexiform layer; *INL* inner nuclear layer; *IPL* inner plexiform layer; *GLC* ganglion cell layer. Scale bar, 20 µm in *upper* panels; 10 µm in *lower* panels

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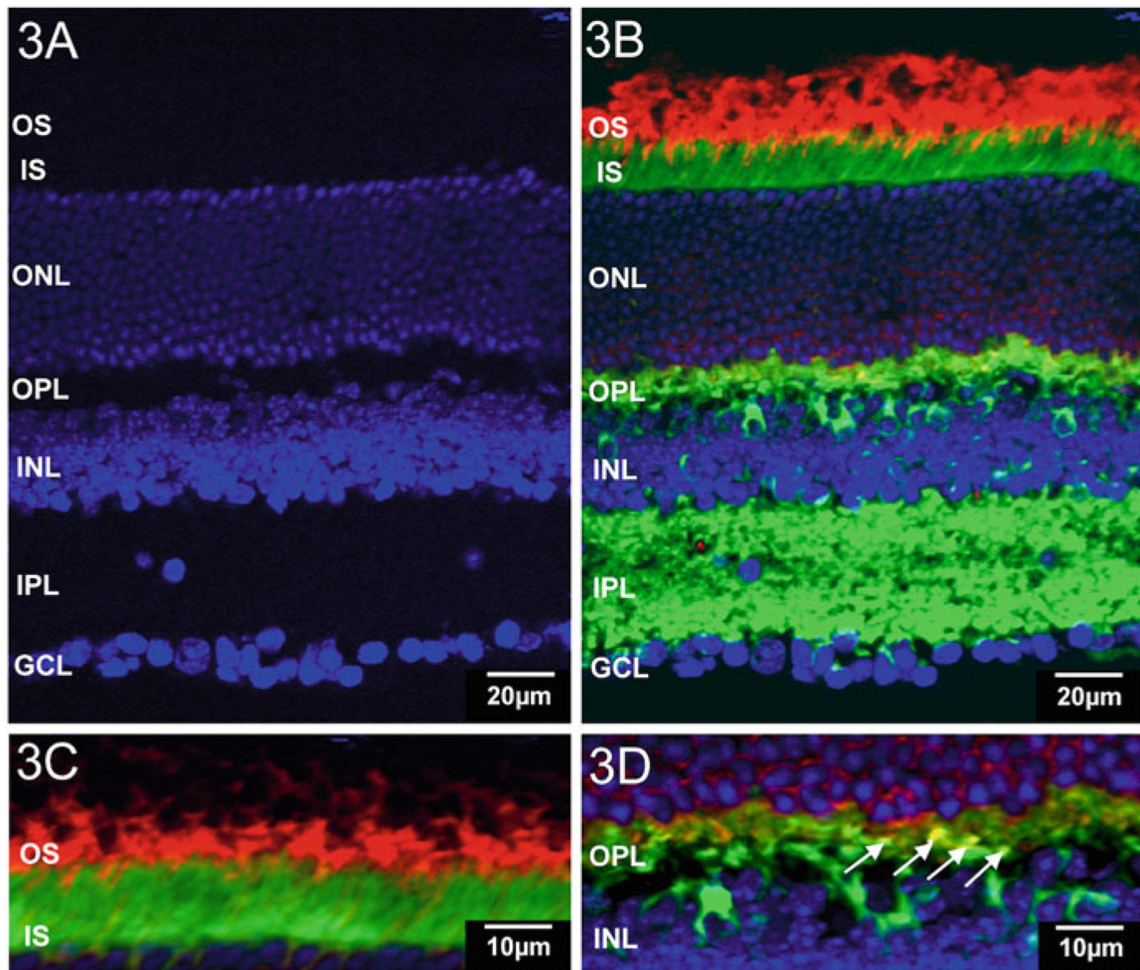

Fig. 101.3.

NSF and Arr1 localization in light-adapted retinas. Immunohistochemical fluorescent labeling of NSF and Arr1 in retinal sections from WT mouse exposed to light for 2 h prior to killing (light-adapted [LA]). Adult WT mouse retina frozen sections were triple labeled fluorescently as in Fig. 101.2 ((**a**, **b**) DAPI stains nuclei blue). NSF immunoreactive staining pattern of NSF is mainly in the OPL and IPL in light-adapted retinas (**c**, **d**). Arr1 immunoreactive pattern is translocated to the outer segment in LA retinas. Arr1 immunoreactivity is extensively dual localized with NSF immunological staining and limited punctate dual staining in the OPL in LA retina ((**d**) higher magnification; *smaller arrows*). Abbreviations are listed in Fig. 101.2. Scale bar, 20 μm in *upper* panels; 10 μm in *lower* panels