1. SPECIFIC AIMS

Tyrosine phosphorylation of intracellular signaling proteins plays an important role in cellular growth, differentiation, proliferation and survival. The initial event in these signaling cascades in non-ocular tissue involves stimulus-mediated activation of a tyrosine kinase. In the mammalian retina several tyrosine kinases have been reported; however, neither their localization to specific retinal cells nor their activation is understood.

The previous specific aims of this proposal were:

1. **Is *c-src* a light-activated tyrosine kinase in rat photorececptor cells/ rod outer segments (ROS) *in vivo*?**

2.What are the identities of the major protein substrates that are tyrosine phosphorylated by light in photoreceptor cells *in vivo*?

3. **Does tyrosine phosphorylation *in vivo* promote the translocation of specific proteins to photoreceptor ROS?**

In the past three years, we have made significant progress investigating the role of the tyrosine kinase Src in rat retina. Our data show that: **1)** Src is a tyrosine kinase that specifically associates with bleached rod outer segment (ROS) membranes, **2)** Tyrosine kinase activity immunoprecipitated with anti-phosphotyrosine from light-adapted ROS (LROS) is twice that recovered from dark-adapted ROS (DROS); moreover, these immunoprecipitates also show the presence of Src in the immune complex, **3)** Src forms a complex in vivo with bleached rhodopsin, rhodopsin kinase (Grk1) and arrestin, **4)** bleached rhodopsin, and arrestin from LROS (but not DROS) are found to associate specifically with immobilized SH2 domain of Src. **5)** transducin " subunit (T") is phosphorylated in vitro by recombinant Src on tyrosine 142, **6)** T" from DROS only is recovered in anti-phosphotyrosine immunoprcipitates and is found to associate specifically with the SH2 domain of Src in vivo **7)** arrestin, Grk1, Src and T" are found to differentially associate with a Triton-X-100- insoluble (cytoskeletal) pellet from ROS in a light-dependent manner, **8)** we have identified caveolin as a novel putative Src substrate in bovine and rat ROS and **9)** We have identified a 110-120 kDa protein that is recognized by an N-terminus peptide-specific antibody to Src on immunoblots which specifically localizes to photoreceptors by immunocytochemistry. Based on these findings, we have focused our efforts towards understanding the detailed mechanism of the light activation of Src and its association with several photoreceptor proteins. Our initial working hypothesis proposed in 1997 (which remains the same) was that light-mediated activation of Src plays a role in the translocation of specific photoreceptor proteins between the inner and outer segments of photoreceptors, or from the cytosol to the disc/plasma membrane/cytoskeleton of photoreceptors. Therefore, understanding in greater detail how Src is activated and how it interacts with bleached rhodopsin, arrestin, Grk1 and T" is crucial to our testing of our hypothesis. We will test this hypothesis by addressing the following specific aims:

1) What is the detailed mechanism of the activation of Src by light?
2) Are transducin " subunit, Grk1 and caveolin in vivo substrates for Src?
3) Is there a photoreceptor-specific Src-like tyrosine kinase in ROS?
4) Does tyrosine phosphorylation of ROS proteins affect the translocation of either transducin " or arrestin?
5) What is the functional outcome on photoreceptors in Src knock-out mice?

Our data indicates the formation of a multimeric complex containing Src, bleached rhodopsin, Grk1 and arrestin (A) that is regulated by light. The primary objective is to determine whether Src interacts directly with bleached opsin, or is coupled through transducin or arrestin, or rhodopsin kinase (Grk1). We propose (FIG.1) that Src in this multimeric complex (as our data indicates) is in an activated form capable of phosphorylating one or more putative substrates in photoreceptor cells (e.g. T", Grk1, or caveolin). Tyrosine phosphorylation of T" would increase its affinity to a protein with an SH2 domain (Src or the tyrosine phosphatase SHP-2), or any protein with a phosphotyrosine binding domain (PTB), thus promoting its binding to disc membranes/cytoskeleton and facilitating its translocation from one cellular compartment to another. Src could also act as an adaptor to Grk1, arrestin or T" and recruit one or more of these proteins from the cytosol to the disc membrane/cytoskeleton. Alternatively, tyrosine phosphorylation of caveolin (a well documented substrate for Src), will provide high affinity binding sites in specialized (cytoskeletal) microdomains for proteins containing either SH2 or PTB domains (Src, SHP-2, PLC(,or PI3-kinase) and facilitate their binding and /or translocation from one cellular compartment to another. Tyrosine phosphorylation of caveolin may also result in its conformational change allowing it to interact with a consensus caveolin binding domain (present in several G-protein signaling components including Src, T" and Grk1) with one or more component of the visual transduction cascade resulting in their membrane recruitment or translocation between the photoreceptor inner and outer segments.

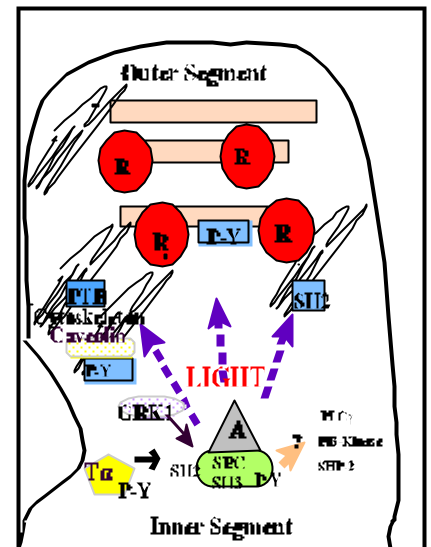

FIG. 1

2. BACKGROUND AND SIGNIFICANCE

Tyrosine Phosphorylation and G Proein-coupled Receptors (GPCR)

In the past few years, several lines of evidence have demonstrated that tyrosine phosphorylation and specific non-receptor tyrosine kinases play important regulatory roles in GPCR signaling (for recent reviews see Hall et al.1999; Gutkind 2000). GPCRs where tyrosine phosphorylation appears to play a role in downstream signaling include the Angiotensin II receptor (AT_1) and the $-adrenergic receptor. Angiotensin II receptor (AT_1), a seven transmembrane receptor with no intrinsic kinase activity, has been shown to induce rapid tyrosine phosphorylation of several intracellular proteins including phospholipase C((PLC(, Marrero et al. 1994, 1995), Shc, mitogen activated protein kinase (MAPK), and focal adhesion kinase, FAK (Tsuda et al. 1991, Molloy et al. 1993, Schorb et al. 1994). Activation of the $-adrenergic receptor has been shown to mediate the activation of MAPK (Koch et al., 1994, Luttrell et al., 1996) as well as tyrosine phosphorylation of the adaptor protein Shc. (Touhara et al., 1995). Other reports have indicated that activation of MAPK involves G$(activation of Src and Src-like non-receptor tyrosine kinases (Luttrell et al, 1996, Igishi and Gutkind 1998).

GPCRs, Arrestins and Src

More recently, a more detailed picture of the activation of Src by the $-adrenergic receptors has emerged. Receptor activation which is usually accompanied by activation of GPCR kinase (Grk), phosphorylation of the receptor and subsequent binding of $-arrestin to the receptor, has been shown to also recruit Src to the membrane resulting in its activation (Luttrell et al., 1999). The trimeric complex of receptor-$-arrestin-Src then mediates tyrosine phosphorylation of dynamin, an essential component of endocytosis. These observations show that $-arrestin acts as an adaptor for Src by recruiting it to the membrane. Other observations have shown that this trimeric complex may involve several different motifs for protein-protein interaction. This interaction was shown to be mediated by the SH3 region of Src binding to a proline-rich region in the N-terminus of β-arrestin not present in visual arrestin. Other reports have shown that β-arrestin as well as visual arrestin interact with the catalytic domain of Src (Miller et al., 2000). Additionally, Src has been recently shown to interact directly with the β_3-adrenergic receptor through its SH3 domain (Cao et al., 2000). Similar to these observations, evidence from our lab (see preliminary results FIG.'s 7 and 8) shows that light exposure in vivo promotes the formation of a complex that contains Src, arrestin, Grk1 and bleached rhodopsin in photoreceptor outer segments, and that activated Src is associated with bleached opsin. Furthermore, tyrosine kinase activity immunoprecipitated from LROS with anti-P-Y antibodies was twice that recovered from DROS (FIG. 5A and B, preliminary results). Studies with immobilized Src SH2 and SH3 domains also demonstrated the association of bleached opsin and arrestin with Src SH2 domain (FIG. 9). These observations would indicate that bleached opsin may interact with Src SH2 domain either directly or indirectly through an adaptor protein. The presence of both rhodopsin, Grk1, and arrestin in immunoprecipitates of LROS with activated Src, suggests a light-dependent formation of a multimeric complex containing rhodopsin, Grk1, arrestin, and activated Src. The specific intermolecular mechanism of association of these proteins remains to be elucidated. Src can putatively interact with other signaling molecules (receptors, adaptor proteins, or enzymes) through its Src homology domains 2 and 3 (SH2 and SH3, respectively), catalytic domain (Miller et al., 2000; Ma et al., 2000), or through its P-Y residues binding to P-Y binding domain (PTB domain, cf. Hubbard et al.,1998 for review). These observations are consistent with earlier observations for the $-adrenergic receptors and clearly suggest several mechanisms for agonist-mediated interaction between Src, arrestins, and GPCRs. The specific module of interaction as well as the functional outcome may depend on cell type, receptor type and stimulus. In specific aim1, we will address the detailed mechanism of light dependent interaction of Src with ROS proteins as well as the specific mechanism of its activation in vivo.

Src and G-protein Alpha subunits

Other recent studies have demonstrated a role for G-protein " subunits in the activation of Src (Ma et al, 2000).In particular, Gs and G"i, but not G$(, G"q, or G"12 can directly stimulate the activity of a downregulated Src. These studies also demonstrate that Gs and G"i bind to the catalytic domain of Src, thus changing its conformation and increasing its accessability to its substrates. Other reports have shown that G" subunits can be substrates for tyrosine kinases in vitro. $G\alpha_s$ (Moyers et al., 1995; Poppleton et al., 1996; Liebmann et al., 1996), $G\alpha_{q/11}$ (Liu et al., 1996; Umemori et al., 1997), and $G\alpha_i$ (Krupinski et al., 1988; Hausdorff et al., 1992; O'Brien et al., 1987) can also be tyrosine phosphorylated by Src. In addition, Src phosphorylated $G\alpha_s$ at Tyr^{37} and Tyr^{377} (Moyers et al., 1995), and $G\alpha_{q/11}$ was phosphorylated at Tyr^{356} (Umemori et al., 1997). We have recently demonstrated that purified bovine T" is phosphorylated in vitro by recombinant Src on Y142. Other in vitro studies have shown that under conditions that promote and preserve P-Y residues, T " is more tightly bound to ROS membranes and is more resistant to extraction by hypotonic GTP washes than unphosphorylated ROS membranes (FIG. 15). Furthermore,

we have observed increased association association with detergent-resistant membranes prepared from bovine ROS that in coincident with increased tyrosine phosphorylation (FIG 16). It is note worthy that recent studies(Seno et al., 2001) confirm our observation regarding the association of T " with detergent-resistant domains. Other in vitro and in vivo experiments (FIG.s 19A and 20 preliminary results, FIG. 10 Bell et. al., 2000) show the association of Tα with Src by co-immunoprecipitation. In specific aim 2, we will determine if T" is phosphorylated in vivo on Y142 (or any other Y residue), and whether this phosphorylation is regulated by light. In addition, the specific mechanism of association of T" with Src will also be investigated.

***Src and Protein-protein Interaction Domains*:**

Src contains several domains besides its catalytic domain capable of interacting with a variety of intracellular signaling molecules (for review see Hubbard et al., 1998). Most notable of these domains are the Src homology regions 2 and 3 (SH2 and SH3, respectively) that have since been identified in a variety of signaling molecules including tyrosine kinases, phosphatases, adaptor proteins, phospholipase C(, phosphatidtylinositol 3-Kinase (PI 3-kinase) and number of other enzymes (for reviews see Pawson and Scott 1997, Hunter 2000). The SH2 domains are able to bind to phosphotyrosine residues (P-Y) with high affinity and specificity with regard to amino acid sequences surrounding the P-Y residue (see Pawson and Scott 1997 for review). The SH3 domain on the other hand interacts with proline-rich sequences with the consensus sequence of PxxPxP (Pawson and Scott 1997). In the appropriate conformation, the SH2 and SH3 domains can interact with either P-Y residues, or proline rich regions of other proteins (Pawson and Scott 1997).This type of interaction has been demonstrated to occur with a variety of receptors, effectors and adaptor proteins (Pawson and Scott 1997, Hunter 2000). Another recently recognized domain is designated PTB (phospho-tyrosine binding domain) which also recognizes P-Y-containing sequences (Forman-Kay and Pawson 1999) that is distinct from those recognized by the SH2 domain. The presence of these domains in diverse proteins with diverse functions allows for the formation of unique signaling complexes upon receptor activation. The specific components of these complexes are dependent on receptor and cell type (Hunter 2000).These complexes have been demonstrated to occur in a variety of GPCRs including ATI binding to SHP-2, PLC(and Jak2 (Marrero et al. 1994, 1995, Venema et al., 1998),β-adrenergic receptor forming a complex with Grb2 (Karoor et al., 1998), and most recently the β-adrenergic receptor forming a complex containing arrestin, Src and the receptor (Luttrell et al., 1999; Miller et al., 2000). One important functional outcome of these complexes is the recruitment of specific signaling molecules to the plasma membrane or other cellular compartment. Our results studying tyrosine phosphorylation in photoreceptor outer segments have also demonstrated the recovery of bleached rhodopsin, arrestin, Src and Grk1 in anti-Src immunoprecipitates(FIG. 8) from LROS, However, more Tα was recovered from DROS immunoprecipitates with anti- PY than LROS (see preliminary results, FIG. 17 B and C). Moreover, we have established that the recovery of these components from DROS and LROS requires interaction of the immobilized SH2 domain of Src with one or more of these components (FIG. 9, FIG.17C). Our first specific aim is to investigate the specific details of this interaction as well as the mechanism of activation of Src by light.

GPCR and Protein Scaffolds:

The localization of signaling proteins to biological membranes is an important mechanism to regulate the specificity and fidelity of signal transduction pathways (Resh 1999). Recent evidence suggests that cellular membranes are not uniform in the distribution of lipids but, instead, contain discrete, detergent-resistant, sphingolipid- and cholesterol-rich microdomains, termed rafts, floating in a sea of detergent-soluble phospholipids (reviewed in Brown and London 2000). The lipid environment of rafts tends to recruit proteins modified with saturated fatty acids (e.g., many signaling molecules including heterotrimeric G protein subunits and Src family tyrosine kinases) while excluding others. Therefore, rafts may act as organizing centers to localize a variety of signaling molecules (Simons and Ikonen 1997).

Caveolins as membrane signal organizers of GPCR.

Caveolae are special, detergent-resistant rafts that contain 21 to 25 kDa integral membrane proteins, caveolins (Rothberg et al. 1992). Caveolin-1 was first identified as a tyrosine-phosphorylated protein in v-Src-transformed fibroblast cells (Glenney 1989). There are three caveolin subtypes: caveolins-1 and -2 are expressed in a variety of cells, including neuronal cells (Galbaiti et al. 1998), and caveolin-3 is expressed predominantly in muscle cells (Tang et al. 1996). Caveolins can form oligomeric complexes within caveolar membranes (Sargiacomo et al. 1995). Furthermore, a wide variety of signaling proteins have been shown to associate with caveolins, including G-protein α-subunits, G-protein-coupled receptor kinases, Src-family tyrosine kinases, and nitric oxide synthases (reviewed in Smart et al. 1999) all of which share motifs that have been suggested to interact with a region of caveolin called the caveolin scaffolding domain (Couet et al. 1997). Direct association of caveolin scaffolding domain-derived peptides with trimeric Gi and Go (Li et al. 1995), c-Src (Li et al. 1996), endothelial nitric oxide synthase (Garcia-Cardena et al. 1997), and phospholipase D1 (Kim et al. 1999) inactivates the intrinsic activities of these proteins. This has led

to the hypothesis that caveolins organize and localize inactive signaling molecules to caveolar membranes (Okamoto et al. 1998).It is noteworthy that T α contains a consensus caveolin-binding domain (a. a. 190-204, Couet et al., 1997) which is berween the switch I and II region (Lambright et al., 1996). This region has been shown to be required for GTP-induced conformational change which is essential for effector recognition (Mittal et al. 1996). Additionally, both Grk1 (Carman et al.1999) and Src also contain a consensus sequence for caveolin binding (Couet et al. 1997) and have been shown to associate with caveolin fusion proteins. We have recently identified caveolin-1 in bovine and rat ROS by immunoblot analysis and immunoprecipitation (see preliminary results, FIG.s18-20). Moreover, a significant amount of caveolin-1 remains associated with a Triton-X-100-insoluble (cytoskeletal) pellet derived from ROS membranes. This is the first observation of this important scaffolding protein which is also a substrate for Src in non-ocular tissue (for review see Anderson 1998, also see below for further discussion).We will address the question of whether caveolin is an in vivo substrate for Src as well as its possible role in membrane recruitment of ROS proteins in specific aims 2 and 4.

Signaling Proteins Phosphorylated by Src

Receptors: From the GPCR family, the AT1 receptor is the only member that has been shown to be tyrosine phosphorylated on Y319 by Src (Venema et al, 1998).On the other hand, the $2-adrenergic receptor has been shown to be phosphorylated by the insulin receptor kinase on tyrosine 350/354 (Hadcock et al., 1992; Karoor et al, 1995, Valiquette et al., 1995; and Baltensperger et al., 1996).***Effectors:*** PLC(1 (Marrero et al., 1994, 1995) , Shp-2 (Venema et al.,1998), G protein receptor kinase2 (Grk2) (Sarnago et al.,1999; Fan et al., 2001), several G-protein " subunits (Moyers et al., 1995; Poppleton et al., 1996; Liebmann et al., 1996 Liu et al., 1996; Umemori et al., 1997) including T" (Bell et al., 2000). ***Adaptors:*** The adaptor proteins Shc (van der Geer et al., 1996) and DOK (Noguchi et al., 1999) and Cbl (Deckert et al., 1998) have also been shown to be substrates for Src family tyrosine kinases. ***Cytoskeletal elements:*** Many components of the cytoskeleton including tubulin (Matten et al.,1990), dynamin (Ahn et al., 1999), gelsolin (De Corte et al.,1999) and caveolin (Nomura and Fujimoto ,1998; Kim et al.,2000; Lee et al., 2000) have been demonstrated to be substrates for Src. Most notable of these is the protein caveolin which is highly enriched in caveolar membranes. These specialized microdomains are resistant to detergent extraction (Anderson et al., 1998).We have recently observed (see preliminary results) tyrosine phosphorylation of caveolin on Y14 in bovine ROS in vitro (under in vitro conditions that promote and preserve P-Y residues) by immunoprecipitation with anti-PY and anti-caveolin(FIG.s 20 and 21). Moreover, we have observed its association in bovine ROS with both Src and T" by immunoprecpitation with anti-Src. Caveolin was also found to be enriched in detergent-resistant pellets (cytoskeleton) obtained from both bovine (FIG.18) and rat ROS (FIG. 22). In other experiments, we have found in vitro evidence for the association of rhodopsin kinase (also designated Grk1) with Src and caveolin in bovine ROS by immunoprecipitation with anti-Src or anti-caveolin (FIG.19). These results are similar to earlier observations regarding the assocaition of Src with Grk2 discussed above (Sarnago et al.,1999 ; Fan et al., 2001) As with other GPCRs, we propose that caveolin may play the role of a scaffold by associating with one or more of the components (e.g. T", or Grk1) of the visual transduction cascade. In specific aim 2, we will address whether T", Grk1and caveolin are in vivo Src substrates and if their phosphorylation is regulated by light. The effect of tyrosine phosphorylation on translocation or membrane recruitment will be addressed in specific aims 4 and 5.

Tyrosine phosphorylation and Photorecepotors

Our initial report of in vivo light-mediated tyrosine phosphorylation of photoreceptor outer segment proteins (Ghalayini et al., 1998a), suggested to us that this protein modification is likey to play an important role in these cells. Although the consequence of light-activation of tyrosine phosphorylation in photoreceptor outer segments remains unknown. Our recent data suggests that Src is a light-activated tyrosine kinase in photoreceptor cells, and may in part be responsible for the increase in tyrosine phosphorylation in ROS membranes. In the past few years, several putative P-Y substrates have been identified in ROS including, PI 3-kinase (Guo et al. 1997), the tyrosine phosphatase, SHP-2 (Bell et al., 1999), transducin α (Bell et al., 2000) and PLCγ1 (Ghalayini et al., 1998b), which binds to bleached ROS membranes *in vitro* and translocates from the inner to the outer segments of photoreceptors in response to light adaptation in vitro (Ghalayini et al., 1998b). The possible regulation of the former enzymes by light in vivo is currently under investigation. A recent report has also demonstrated tyrosine phosphorylation of the EGF and FGF receptors in response to EGF and FGF treatment of photoreceptor cells in culture (Fontaine et al., 1998). In non-ocular tissue, the EGF receptor has been shown to associate with Src, and to be regulated by it (Biscardi et al., 1999). Other reports have shown that the photoreceptor cGMP-gated channel is modulated by tyrosine phosphorylation (Molokanova et al., 1997, 1999). The cumulative evidence (8 reports from 4 different laboratories) clearly point to the potential importance of this complex pathway for processes ranging from modulation of photoresponse to cell survival.

Functional and Structural polarization of Photoreceptor cells

The photoreceptor cell is polarized structurally and functionally. There is significant evidence that the maintenance of this functional polarization is regulated by light. This is illustrated by the translocation of specific signaling components between the inner and outer segments of photoreceptors in a light-dependent manner. This includes arrestin (Broekhuyse et al., 1985, Whelan and McGinnis 1988), transducin (Brann and Cohen 1987), and phosducin (Whelan and McGinnis 1988). Maintenance of a molecular equilibrium between these components of the visual transduction cascade is likely to play a role in photoreceptor adaptation or turnoff. The translocation of arrestin and transducin between the inner and outer segments in response to light is well documented. Mechanistically, the transport/ translocation of these components within the photoreceptor is completely unknown. ***For example, how does arrestin translocate from the inner segment to the outer segment within minutes of light exposure? Additionally, how are T'' and Grk1 (rhodopsin kinase) predomimamtly cytosolic components (in the dark) of the visual transduction cascade recruited to the disc membranes for interaction with bleached rhodopsin? What is the signal that brings these molecules from the cytosol to the membrane?There are two distinct processes at work in these movemements: 1) traslocation between distinct comparments of soluble signaling proteins, and 2) recruitment of the same signaling components to the disc membranes for interaction with bleached rhodopsin.*** Currently, neither process is clearly understood. However, our recent observations may provide some clues. In preliminary results we present evidence for the association of Src, Grk1, arrestin and transducin with ROS cytoskeletal fractions. Moreover, Src, Grk1 and arrestin are all enriched in LROS cytoskeleton over DROS cytoskeleton, while the reverse is true for T''. In other experiments, we also present evidence for the presence of caveolin-1 in both D and LROS cytoskeleton. The association of arrestin and Grk1 which are both predominantly soluble proteins with a detergent-resistant pellet in the light might suggest a role for the photoreceptor cytoskeleton in this movement. The same argument would also be applicable for T'' association in the dark. In specific aim 4, we will address the role of Src and caveolin in mediating the association of arrestin, transducin and Grk1 with the cytoskeleton as well as the role that activation of Src plays in the translocation or membrane recruitment of these proteins. In specific aim 5, we will directly test the role of Src in membrane recruitment or protein translocation using a Src knock-out mouse model. ***Summary:*** Our latest observations show that: 1) tyrosine kinase activity associated with LROS found in immunoprecipitates with anti-P-Y which also contain Src is twice that obtained from DROS, 2) several cytosolic ROS proteins (Arrestin and Grk1) in addition to bleached rhodopsin are found to co-immunoprecipitate with anti-Src in a light-dependent manner suggesting that one or more of these components associates with Src. In addition, this association appears to require interaction with the SH2 domain of Src, indicating that the protein in this complex directly responsible for the interaction with Src is tyrosine phosphorlated; 3) several cytosolic components (arrestin, Grk1 and T'') in addition to Src are also associated with the photoreceptor ROS cytoskeleton in a light-dependent manner, and 4) caveolin, a cytoskeletal component documented to be a Src substrate and to interact with several signaling components of GPCRs through its signature caveolin domain is also present in ROS cytoskeleton. As shown (FIG. 2), Src is a multifunctional tyrosine kinase that can interact with other signaling molecules through several of its domains (SH2, SH3, P-Y, discussed earlier) as well as its catalytic domain (Miller et al., 2000; Ma et al., 2000). Therefore, in addition to its intrinsic kinase activity, it can play the role of an adaptor or coupling protein through its ligand-specific domains.

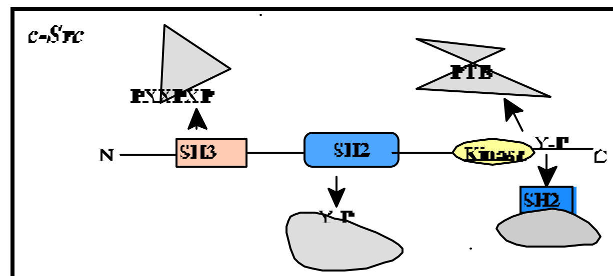

FIG. 2. Src contains several domains (SH2, SH3, catalytic and P-Y) that are capable of interacting with specific motifs in other signaling proteins; SH2, SH3; Src homology region 2 and 3, respectively; PXXPXP, proline-rich domain; PTB; phosphotyrosine binding domain and P-Y: phosphotyrosine.

Based on these recent findings, the hypothesis we propose can be summarized as follows:

Light° Rhodopsin° activation of tyrosine kinase (Src) in the inner segment° tyrosine phosphorylation of other cytosolic photoreceptor proteins in the inner segment° direct recruitment/transport of cytosolic signaling molecules to ROS (e.g. T'', and Grk1 both putative in vivo substrates for Src), or recruitment/transport of cytosolic proteins (e.g. arrestin) through their association with an adaptor molecule that is tyrosine phosphorylated (FIG. 1). P-Y residues on these cytosolic proteins bind to SH2 or PTB (phosphotyrosine binding domain) binding domains present in an integral ROS protein at the base of the outer segment or a cytoskeletal element present throughout the photoreceptor, which ultimately leads to their transport from the inner to the outer segment. Alternatively, Light° Rhodopsin° activation of tyrosine kinase (Src)° translocation/membrane association of Src with ROS° tyrosine phosphorylation of an integral ROS membrane protein/cytoskeletal component (e.g. caveolin) providing high affinity binding sites (P-Y) for signaling proteins with either an SH2 domain (e.g. PLCg, PI 3-kinase, SHP-2, all present in ROS), or a phosphotyrosine binding (PTB)

all present in ROS), or a phosphotyrosine binding (PTB) domain. Additionally, caveolin phosphorylation could lead to a conformational change that alters its interaction with proteins that contain a caveolin binding domain (Grk1, T[illegible], and Src). In either model, P-Y residues generated in a light-dependent manner provide a module for protein-protein interaction between cytosolic photoreceptor proteins and integral membrane/cytoskeletal components of ROS. This module provides docking sites for transport of cytosolic proteins between the inner and outer segment either through association with the cytoskeleton or an integral ROS protein. In addition, this module may also provide a mechanism for the recruitment of cytosolic components within the OS to disc membrane. Depending on the identity of the signaling protein recruited, the outcome will either modulate the photoresponse (light adaptation/turn-off, e.g. Grk1 or arrestin translocation), or contribute to other light-regulated (entrained) events that are important for the maintenance of structural integrity of photoreceptor cells (disc shedding/morphogenesis).