Guanylate cyclases and associated activator proteins in retinal disease

David M. Hunt · Prateek Buch · Michel Michaelides

Abstract Two isoforms of guanylate cyclase, GC1 and GC2 encoded by *GUCY2D* and *GUCY2F*, are responsible for the replenishment of cGMP in photoreceptors after exposure to light. Both are required for the normal kinetics of photoreceptor sensitivity and recovery, although disease mutations are restricted to *GUCY2D*. Recessive mutations in this gene cause the severe early-onset blinding disorder Leber congenital amaurosis whereas dominant mutations result in a later onset less severe cone–rod dystrophy. Cyclase activity is regulated by Ca^{2+} which binds to the GC-associated proteins, GCAP1 and GCAP2 encoded by *GUCA1A* and *GUCA1B*, respectively. No recessive mutations in either of these genes have been reported. Dominant missense mutations are largely confined to the Ca^{2+}-binding EF hands of the proteins. In a similar fashion to the disease mechanism for the dominant *GUCY2D* mutations, these mutations generally alter the sensitivity of the cyclase to inhibition as Ca^{2+} levels rise following a light flash.

Keywords Phototransduction · Retinal dystrophy

Retinal-specific guanylate cyclases (GCs) and their associated activator proteins (GCAPs) are responsible for the Ca^{2+}-sensitive restoration of cGMP levels after the light activation of the phototransduction cascade. These proteins play a central role in phototransduction (Fig. 1) and it is

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perhaps not surprising that mutations in the corresponding genes result in blinding disorders.

Guanylate cyclases

Early studies showed that two forms of guanylate cyclase are present in rod and cone photoreceptors [1–3]. Both show inhibition by Ca^{2+} (reviewed in [4, 5]) via interaction with GCAPs. GCAPs bind constitutively to GCs; in the absence of Ca^{2+}, they stimulate cyclase activity whereas in the presence of Ca^{2+}, they are inhibitory. The human forms (retGC1 and retGC2) are encoded by the genes *GUCY2D* and *GUCY2F* but are referred to as *ROS-GC1* and *ROS-GC2* in bovine and *GC-E* and *GC-F* in mouse. In this review, the notation of *GC1* and *GC2* will be adopted. Both genes comprise 18 coding exons plus a 5′ and a 3′ non-coding exon that specify proteins with predicted functional domains that consist of an extracellular or intradiskal domain, a transmembrane segment, a kinase homology domain, a dimerization domain and a catalytic domain (Fig. 2a). Sequence motifs within the kinase homology domain are important for interactions with GCAPs [6, 7] although the critical region for this lies within the catalytic domain [8–11]. *GC1* is detected in the retina, pineal gland, olfactory bulb, cochlear nerve and organ of Corti [12–14], whereas *GC2* is found only in the retina. cGMP synthesis requires dimerization to create the catalytic site of the cyclase and this is achieved by an α-helical coiled-coil structure that maintains the interaction between subunits [15] (Fig. 2b). Heterodimers between *GC1* and *GC2* subunits can be formed in vitro, although experiments with retinal tissue indicate that homodimers predominate [16].

The relative roles of *GC1* and *GC2* in phototransduction have been clarified largely through the study of mouse
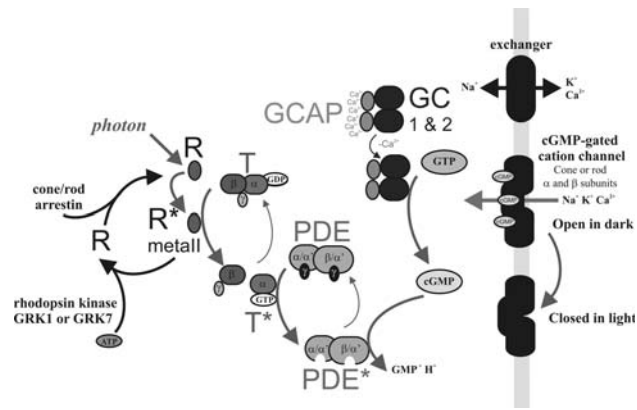
Fig. 1 The phototransduction cascade. Guanylate cyclase *GC*, guanylate cyclase activating protein *GCAP*, phosphodiesterase *PDE*, rhodopsin *R*, transducin *T*

mutants with null mutations in *GC1* and *GC2*, respectively. Disruption of *GC1* while leaving *GC2* intact [17] had little effect on rod numbers or rod morphology but increased the rate of recovery of rods from a light flash. In contrast, cones appeared initially unaffected, but had disappeared by 5 weeks of age [17, 18]. Scotopic electroretinogram (ERG) a- and b-wave amplitudes were reduced in $GC1^{-/-}$ mutant mice but were completely absent in $GC1^{-/-}$ $GC2^{-/-}$ double knockout mice, confirming that no other guanylate cyclase is present that is functional in retinal phototransduction. Delayed recovery of the a-wave in $GC1^{-/-}$ and $GC2^{-/-}$ mice indicates that both contribute to the recovery of photoreceptors after exposure to intense bleaching. Dark currents of $GC1^{-/-}$ and $GC2^{-/-}$ mice were not significantly different from wild type (WT); the basal activity of either *GC1* or *GC2* is sufficient therefore to maintain dark current in rods. The sensitivity of $GC2^{-/-}$ rods was similar to WT whereas the sensitivity of $GC1^{-/-}$ rods was about 3.5 fold higher than WT. These findings suggest an important physiological role of both GC1 and GC2, with neither alone being able to support normal phototransduction. It is therefore surprising that mutations in *GC2* associated with retinal disorders have yet to be identified.

Recessive mutations in *GC1*

Recessive mutations in *GC1* are a major cause of Leber congenital amaurosis (LCA). It is now recognised that LCA represents the most severe end of the spectrum of infantile onset rod–cone dystrophies. This has led to the use of the term early onset severe retinal dystrophy (EOSRD) to describe this group of disorders. LCA was first described by Theodore Leber in 1869 [19], and although it has a population frequency of only about 1 in 30,000, it is a major cause of blindness in children [20]. It presents at birth or soon after with severe visual loss, sensory nystagmus, reduced or absent papillary response and a flat ERG. Visual acuity ranges from 20/200 to light perception, with progression not being a feature of all EOSRD phenotypes (Fig. 3). The underlying genetic cause is heterogeneous and the associated phenotype varying with the gene involved. Recessive mutations in 14 different genes have now been described [21]. However, the first gene to be associated with LCA was *GC1* [22]. Recessive mutations in *GC1* are one of the most common causes of LCA, with a variable frequency ranging from 21% [23] to 12% [24] of all LCA cases depending on the population studied. Mutations are scattered across the gene, with reported mutations in most coding exons, in several introns, and in the 5′-UTR [22, 23, 25–32]. A post-mortem study [33] of

Fig. 2 **a** Exons of *GC1* gene and corresponding protein domain structure. **b** Schematic model of the regulation of wild-type GC1 by GCAP1 under light and dark conditions. Maximal activation occurs only at free Ca^{2+} concentrations below 100 nM, and above 1 μM < 10% activity persists. **c** Arg838 mutant GC1 alters the position of the equilibrium as a result of the increased stability of the coiled-coil structure holding the activated dimer together. In consequence 60% of maximal activation by GCAP1 persists at high Ca^{2+} concentrations
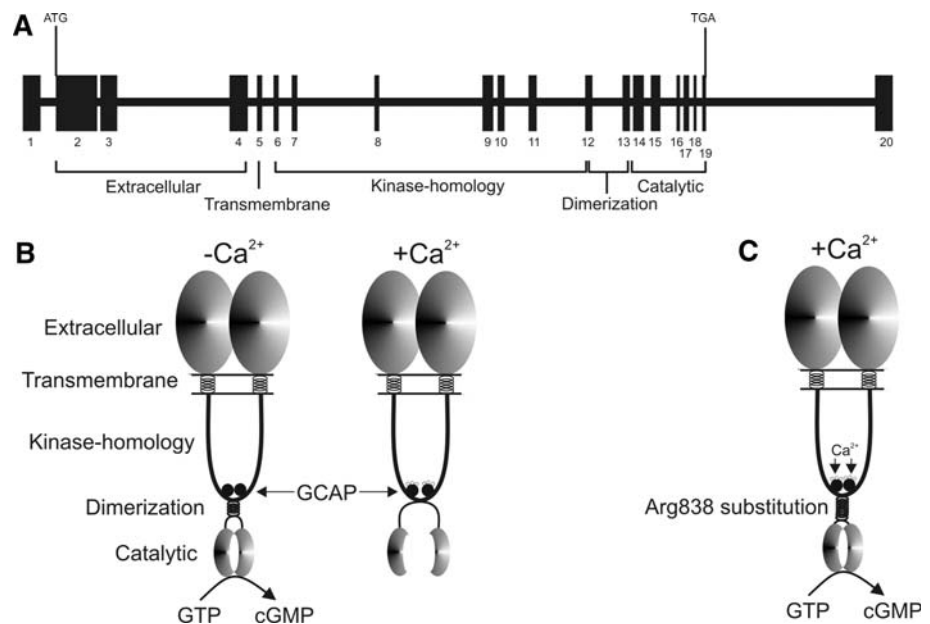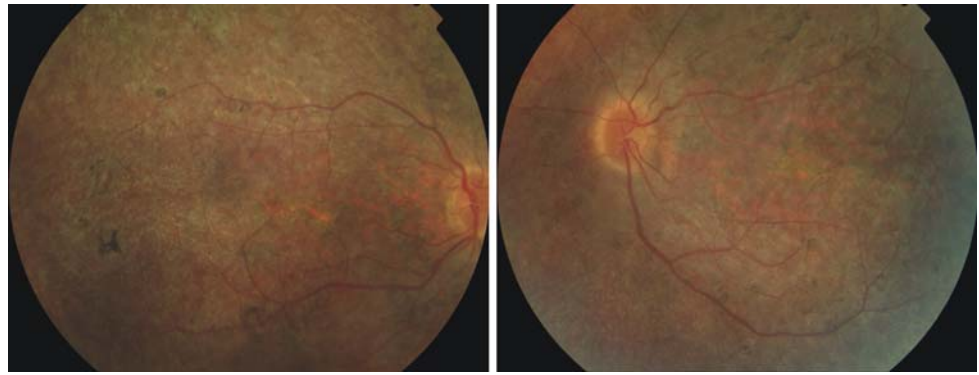
Fig. 3 Fundus photographs of late stage LCA showing pigmentary retinopathy, macular atrophy and retinal arteriolar narrowing

the eye of a patient diagnosed with LCA associated with a missense mutation in one allele of *GC1* and a putative deletion in the other demonstrated that rods and cones, although absent in the mid-periphery of the retina, were still present in the macular and far periphery of the retina. These photoreceptors lacked outer segments. Cones formed a monolayer of cells bodies but rods were clustered and had sprouted neurites in the periphery. The presence of significant numbers of residual photoreceptors is encouraging for the potential success of future gene therapy-related treatment studies.

Many of the LCA-1-causing *GC1* mutations are nonsense mutations that if translated would encode truncated proteins which would lack domains critical to normal function, although the intervention of nonsense-mediated mRNA decay would most likely mean that no protein is produced, resulting in the total absence of cyclase activity. There are nevertheless several missense mutations; those within the catalytic domain show severely reduced or absent cyclase activity as a direct interference with the catalytic site [34–37] whereas those within the extracellular domain have only a moderate effect on cyclase activity [37] (Fig. 4). It is presumed in the latter cases that misfolding of the mutant protein results in degradation and thereby loss of activity [36, 37].

A dominant negative effect of the Pro858Ser and Leu954Pro mutations has been demonstrated in in vitro activity studies [37]. Both mutations severely impair basal and GCAP-stimulated catalytic activity of the enzyme, and when co-expressed with a WT allele, show a reduced activity of GC1. This is consistent with a dominant negative effect as indicated by cone ERG abnormalities in heterozygous carriers of the Leu954Pro mutation [20].

A novel mutation in *GC1* underlies an early onset form of retinitis pigmentosa which presents as a severe rod–cone dystrophy with progressive high myopia and macular preservation [38]. The disorder was shown to arise from a homozygous 4 bp insertion in exon 19 of *GC1*, a mutation which would be expected to result in a 28 amino acid elongation of the protein. The cyclase generated by this recessive mutation may not therefore lack activity as expected for typical LCA-1 mutations. However, when present in the heterozygous state with a more typical *GC1* null mutation, it nevertheless gave rise to a typical LCA-1 phenotype [38].

Animal models and treatments

The *GC1* knock out mutation in the mouse results in a null allele with a partially deleted exon 5 [18]. It would appear to be a good genetic model for LCA since in both mutant mice and human patients, activity of GC1 is either absent or severely curtailed. Results show that the rate of cone cell loss in the $GC1^{-/-}$ null mutant mice is comparable to that found in humans with LCA [18, 39]. Cones are still abundant at 2 months but show a gradual decrease. They have highly disorganized outer segments [17], reduced levels of cone opsins, and lack cone transducin (α and γ subunits), cone α′ phosphodiesterase and the G-protein-coupled receptor kinase 1 [17]. The light dependent translocation of cone arrestin was also absent but could be restored by gene therapy treatment of $GC^{-/-}$ mice with an AAV-GC1 construct [40], indicating a role for GC1 in the trafficking of these proteins within photoreceptors. The translocation of rod arrestin and rod α-transducin in rod photoreceptors were, however, not affected [41]. These observations indicate a severe cone dystrophy which does not extend to the rods, unlike LCA/EOSRD in humans. Rods appear morphologically normal, although they do display altered responses to light [18].

The *retinal degeneration* (rd) mutation in chickens, first identified in Rhode Island Reds [42], arises from a deletion/ insertion mutation in the avian *GC1* orthologue. In this mutation, exons 4–7 are replaced by an 81-bp fragment with 89% sequence identity to a portion of exon 9 in reverse orientation [43, 44]. The reading frame is not disrupted by this event but the mutant protein would be predicted to lack the membrane-spanning domain and flanking regions; it is unlikely that any cyclase activity would be retained. This chicken mutant thereby shares a common
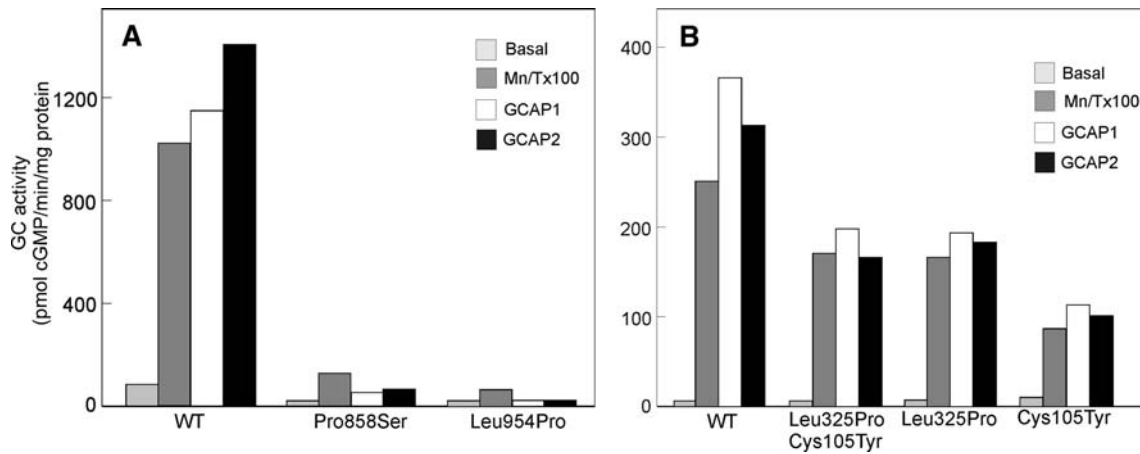
Fig. 4 Recessive LCA-1 mutations. **a** Basal and stimulated in vitro activity of WT and mutant GCs with Pro858 and Leu954Pro catalytic domain mutations. **b** Basal and stimulated in vitro activity of WT and mutant GCs with Cys105Tyr and Leu325Pro extracellular domain mutations. Mutant GCs were expressed and analysed in vitro either singly or in combination. Redrawn from [37]

genetic basis therefore to LCA-1 in man. At hatching the retinae of affected chicks appear morphologically normal but no ERG can be measured under either light- or dark-adapted conditions [42]. Rods and cones of affected animals undergo degenerative changes after about 1 week post-hatching, with clear signs of reduced and disorganized outer segments and photoreceptor loss by 21 days, in keeping with human LCA/EOSRD. By 6 months, very few photoreceptor inner segments and nuclei remain. Pathological changes were frequently noted in the pigment epithelium overlying the degenerating retina. Recent trials with a lentivirus-based gene transfer vector carrying the *GC1* gene injected into early-stage mutant embryos showed a partial restoration of ERG responses and a slowing in the rate of retinal degeneration [45].

Dominant mutations in cone and cone–rod dystrophies

The cone and cone–rod dystrophies are both clinically and genetically heterogeneous. They represent a group of progressive retinal disorders which are an important cause of blindness in childhood and early adulthood [46]. The first dominant mutation in *GC1* was identified in an extended family (designated as CORD6) with a severe cone–rod dystrophy (CORD) [47] in which linkage analysis had previously mapped the disease gene to chromosome 17p12–p13 [48]. CORD in this family displayed an early onset, with marked loss of central vision in the first decade and peripheral field loss by the fourth decade. Affected subjects often had photophobia, nystagmus and myopia. Fundoscopy revealed a 'bull's eye' maculopathy early in the disease, with later involvement of the peripheral retina (Fig. 5). ERGs showed no detectable cone responses early in disease, with progressive abnormality of rod responses appearing later [49–51]. The ERG was often
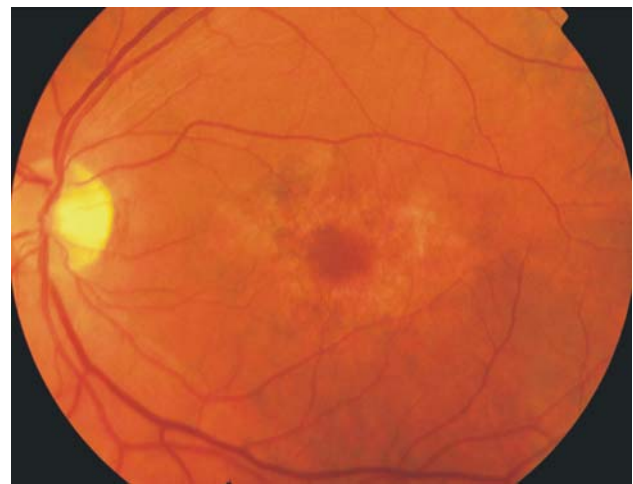

Fig. 5 Fundus photograph showing a bull's eye maculopathy that may be seen in CORD associated with *GC1* mutations

'electronegative', suggesting early involvement of inner retinal neurons.

Two heterozygous missense mutations in adjacent codons were identified in the CORD6 family that encode Glu837Asp and Arg838Ser substitutions [47, 52]. A second CORD family was subsequently identified [53] with changes in three adjacent codons that result in Glu837Asp, Arg838Cys, Thr839Met substitutions. The disease-causing change is, however, most likely the substitution at site 838 as subsequent CORD patients identified with mutations in *GC1* possess only a codon 838 change (Table 1).

Mutations in *GC1* are a major cause of dominant cone–rod and cone dystrophies, accounting for up to 35% of all such cases [51, 52, 54]. With one exception, all are confined to codon 838. The exception is a mutation in exon 8 that encodes a Pro575Leu substitution within the kinase-homology domain of the cyclase [55]. The clinical

Table 1 GC1 mutations in dominant retinal disease

Mutation	Reference
Glu837Asp; Arg838Ser	[47]
Arg838Cys	[47], [94]
Arg838His	[52], [95]
Glu837Asp; Arg838Cys; Thr839Met	[53]
Pro575Leu	[55]

phenotype of this disorder is more accurately described as a dominant cone dystrophy as scotopic (rod) function was normal but photopic function was generally absent.

Mutations at site 838 of *GC1* have been subjected to functional analysis of cyclase activity [56, 57]. When modelled in vitro, the Arg838 substitutions result in an increase in the apparent affinity of GC1 for GCAP-1 and a decrease in the Ca^{2+} sensitivity of the GCAP-1 mediated response, thereby allowing the catalytic activity of the mutant protein to be stimulated by GCAP-1 at higher Ca^{2+} concentrations than WT (Fig. 6). Stimulation by GCAP-2 is also dramatically reduced [56]. The effect of substitutions at site 838 on the Ca^{2+} sensitivity of the GCAP1 activation increases from WT < R838C < R838H < R838A < R838S [57]. This is consistent with the report that patients with Arg838Cys or Arg838His substitutions have milder clinical phenotypes that the original CORD6 patients with a Arg838Ser substitution [46, 49].

The cyclase activity of GC1 requires dimerization to form the catalytic site [15]. This dimerization requires an α-helical coiled-coil structure between the dimerization domains of the two subunits (Fig. 2b). The strength of this coiled-coil structure must be strictly regulated for normal
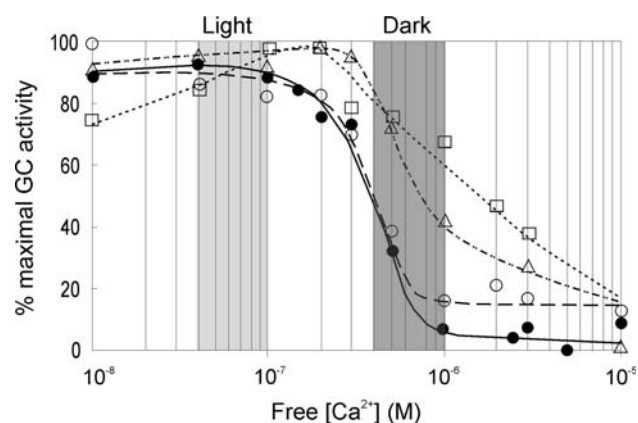

Fig. 6 In vitro wild type and mutant GC1 activity at varying free Ca^{2+} concentrations. Activities are presented as percentages of maximum GC1 activation. WT: *filled circles*, *solid line*. Arg858Cys: *open circles*, *dashed line*. Arg858Ser: *squares*, *dotted line*. Arg858His: *triangles*, *dotted* and *dashed line*. *Shaded boxes* indicate Ca^{2+} concentrations in light and dark. Re-drawn from [57]

interaction with GCAP1; this is altered by substitutions at site 838 which disrupt a small network of salt bridges and allows an abnormal extension of the coiled-coil structure (Fig. 2c) and thereby a reduction in Ca^{2+} sensitivity of the cyclase [15, 57]. An alternative explanation is that these substitutions cause a change in the relative affinities of the mutant cyclases for Ca^{2+}- and Mg^{2+}-bound forms of GCAPs [58]. In either case, mutant cyclase activity would be maintained at high physiological intracellular Ca^{2+} concentrations.

Guanylate cyclase activating proteins

The guanylate cyclase activating proteins belong to a family of neuronal Ca^{2+} sensors. In many mammalian species including human, chimpanzee, macaque and dog [59], three forms of GCAP have been identified (GCAP1 encoded by *GUCA1A*, GCAP2 encoded by *GUCA1B* and GCAP3 encoded by *GUCA1C*) but only *GCAP1* and *GCAP2* are present in the mouse genome, *GCAP3* being represented as a pseudogene [60]. Immunocytochemical studies in mice have shown that GCAP1 is expressed in rods and cones whereas GCAP2 is predominately expressed in rods and may also be present in cells of the inner retina [61–63]. In lower vertebrates, additional copies may be present, with up to eight different *GCAP* genes in teleosts [60, 64]. In humans, GCAP1 and GCAP2 are expressed in rod and cone photoreceptors, whereas GCAP3 is expressed in cones only [60, 65].

Immunolabelling studies [66] have shown that GCAP1 is predominantly present in cone outer segments with only sparse and low level labelling in rod outer segments. In contrast, GCAP2 is more concentrated in rods than cones, although the synaptic regions of both types of photoreceptors were labelled. The different localizations of GCAPs suggest that the roles of GCAP1 and GCAP2 may be different.

The genes for GCAP1 (*GUCA1A*) and GCAP2 (*GUCA1B*) are arranged in a tail-to-tail array on human chromosome 6p21.1 [67] and mouse chromosome 17 [62] whereas GCAP3 (*GUCA1C*) is on human chromosome 3q13.1. The GCAP proteins contain four EF hands that each comprise a helix-loop-helix conformation, with EF1 modified for interface with GCs [68], leaving EF2-4 for Ca^{2+} binding (Fig. 7). These loops have canonical Ca^{2+} binding sites formed by acidic residues for Ca^{2+} coordination. EF1 and 2 are contained within the N-terminal domain of the protein whereas EF3 and 4 are within the C-terminal region [69]. Many members of the family of neuronal calcium sensors have a 'calcium-myristoyl switch' where a myristoyl group is buried inside the protein in the Ca^{2+}-free state but becomes fully exposed on Ca^{2+}-
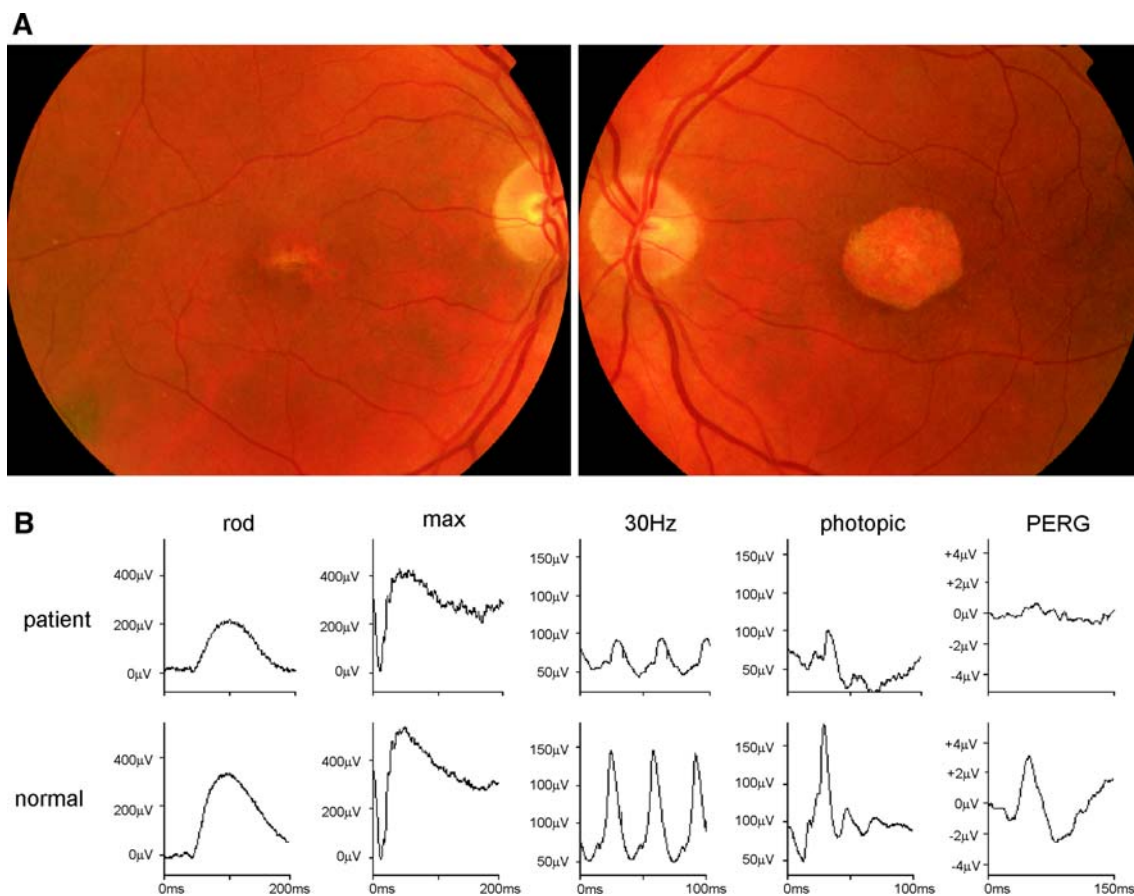
Fig. 7 **a** Fundus photographs showing bilateral macular atrophy, more marked in the left than right eye. **b** Typical electrophysiological findings seen in isolated cone dystrophy. The rod specific and maximal ERGs fall within the normal range. The 30 Hz flicker ERG is both delayed and reduced, typical of most cone dystrophies. The photopic single flash ERG is markedly subnormal with particular reduction in the b-wave. However, it is of note that cone single flash and flicker ERGs often have minimal implicit time shift (delay) in subjects harbouring GCAP1 Tyr99Cys mutations [96]. The pattern ERG (PERG) is profoundly subnormal in keeping with marked macular involvement. Figure kindly provided by Professor Graham E. Holder, Moorfields Eye Hospital

binding. However, GCAP2 has been shown to lack a Ca^{2+}-myristoyl switch [70, 71] and recent studies on the conformational structure of GCAP1 indicates that the myristoyl group remains fully buried in both the Ca^{2+}-free and Ca^{2+}-bound state [72].

The relative roles of *GCAP1* and *GCAP2* have been largely determined by studies with mouse knockout mutations. In mice in which both genes have been disrupted, GC activity lacks Ca^{2+} dependence, confirming the role of GCAPs in the Ca^{2+} regulation [73]. In these mice, flash responses from dark-adapted rods are larger and slower than WT, and incremental flash sensitivity of rods fails to be maintained at a WT type level. Restoration of GCAP2 production via a transgene restores maximal light-induced GC activity but does not restore normal kinetics to responses evoked by saturating flashes [73]. In contrast, the provision of GCAP1 via a transgene shows that the degree of recovery of the rod a-wave is correlated with the level of expression of GCAP1. In single cell recordings, the majority of rods generated flash responses identical to WT [74].

A more direct study of the role of GCAP2 on phototransduction has been carried out in mice with a disrupted *GCAP2* gene but retaining a fully functional *GCAP1* gene [75]. In these mice, rod viability and outer segment morphology appeared normal, and there were no compensatory changes in the levels of GC1, GC2 or GCAP1. However, there was a two-fold drop in the maximal rate of cGMP synthesis at low Ca^{2+} concentrations, and an increase in the Ca^{2+} concentration at which the half-maximal rate of cGMP synthesis was achieved. Flash responses recovered more slowly than WT and rods were more sensitive to flashes but tended to saturate at lower intensities. GCAP2 is necessary therefore for normal recovery of rods and for light adaptation; for the rapid activation and deactivation of GC activity in response to light, both isoforms of GCAP are required.

Cone responses are also affected by GCAP-knockout mutations. Under cone isolation conditions, ERGs recorded from mice lacking both GCAP1 and GCAP2 had normal amplitudes of the saturated a-wave and b-wave [76]. However, the b-wave was widened, there was an increase in the sensitivity of both M- and UV-cones, and the recoveries of the cone-driven a-wave and b-wave were delayed. Restoration of GCAP1 via a transgene showed that recovery of the cone-driven a-wave was restored to normal, although the recovery of the cone-driven b-wave was slightly faster than that observed in WT mice. These studies reveal that the absence of GCAP1 and GCAP2 delays the recovery of light responses, and that GCAP1 restores the recovery of cone responses in the absence of GCAP2 [76], suggesting a more important role for GCAP1 in cone phototransduction, consistent with the proposal that GCAP1 is more highly expressed in cones than in rods [66].

Mutations in *GCAPs*

No mutations in *GCAP3* have so far been found in retinal disease [77]. A dominant Gly157Arg missense mutation in *GCAP2* was identified in three independent families with incomplete penetrance and a spectrum of disease phenotypes that included retinitis pigmentosa (RP), RP with macular involvement and macular degeneration [78]. The first reported mutation in *GCAP1* was a missense mutation in codon 99 that results in the replacement of a highly conserved Tyr with Cys (Tyr99Cys) [79]. Other reported mutations are listed in Table 2. The clinical phenotype for Tyr99Cys patients was initially reported as an isolated progressive cone dystrophy [79] and this is a feature of many of the other *GCAP1* mutations [46], although a recent clinical re-evaluation of the original family with the Tyr99Cys mutation [80] found a range of phenotypes that included reduced cone and rod responses (with cone loss greater than rod, in keeping with CORD), and isolated macular dysfunction (Fig. 7). This demonstrates the extent of intrafamilial heterogeneity in retinal dysfunction that

may be present in persons with the same gene mutation. All affected subjects complained of mild photophobia and reduced central vision and colour vision. Onset is between the third and fifth decade, with subsequent gradual deterioration of visual acuity and colour vision.

The Tyr99Cys substitution (Fig. 8) is located in the helical region of EF3 and does not interfere with the ability of mutant GCAP1 to activate GC1 at low Ca^{2+} concentrations but does prevent Ca^{2+}-bound mutant GCAP1 from inhibiting GC1, thus making GC constitutively active at physiological Ca^{2+} concentrations. Tyr99 is part of a hydrogen bond network that includes Ser173 and Tyr55; the Tyr99Cys substitution would disrupt this network and thereby destabilize the specific inter-domain orientation of the Ca^{2+}-bound GCAP [69]. Another mutation that is reported to cause a cone dystrophy results from an Asn104Lys substitution in EF3 [81].

The Glu155Gly substitution in the loop region of EF4 replaces the negatively charged Glu residue that is directly responsible for coordinating Ca^{2+} with an uncharged residue, Gly. Two other sites in EF4 where mutations have been reported are Leu151 [82, 83] and Ile143 [84]. The
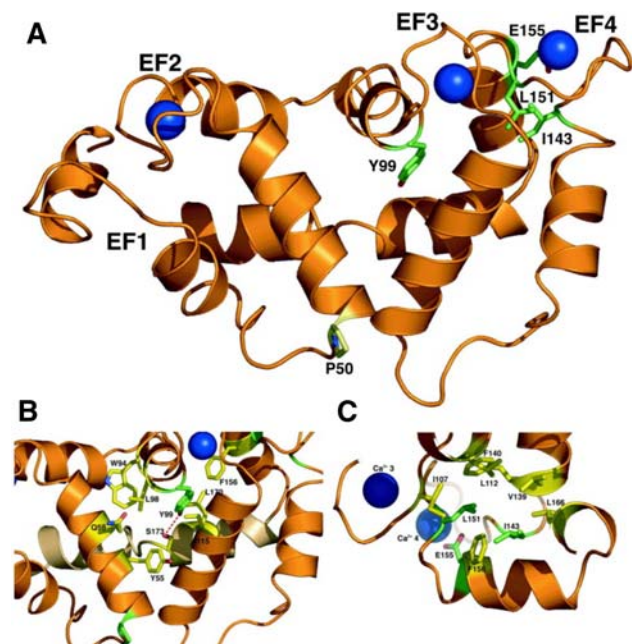
Table 2 GCAP1 mutations in dominant retinal disease

Mutation	Domain	Dystrophy	Reference
Pro50Leu		Cone	[90]
Glu89Lys	EF3	Cone	[89]
Tyr99Cys	EF3	Cone-rod	[79]
Asp100Glu	EF3	Cone	[89]
Asn104Lys	EF3	Cone	[81]
Ile143AsnThr	EF4	Cone	[84]
Leu151Phe	EF4	Cone-rod	[82, 83]
Glu155Gly	EF4	Cone	[88]
Gly159Val	EF4	Cone	[89]

Fig. 8 GCAP1 model based on the crystal structure of GCAP3. **a** Mutated residues Tyr99, Ile143, Leu151, Glu155 which alter Ca^{2+} sensitivity are coloured in *green*. Residue Pro50 where mutation appears to reduce stability is shown in *yellow*. **b** Close up of residues around residue Tyr99 showing the hydrogen bonding interaction with Ser173 from the kinked C-terminal helix (coloured *orange*). **c** Close up of the Ca^{2+}-binding loops of EF 3 and 4 showing the highlighting the Ca^{2+}-coordinating function of Glu155 and the hydrophobic pocket that accommodates residues Ile143 and Leu151. Note color is only available in the online version of the figure. Reproduced from [69] with permission

former is a simple point mutation generating a Leu151Phe substitution, but the latter is a complex change involving a single bp deletion in codon 143 followed by a 4 bp insertion which results in the substitution of Ile by Asn and the insertion of Thr (Ile143AsnThr) [84]. This mutation was identified in two patients (father and son) with dominant cone degeneration. A histopathological evaluation of the father's eyes at autopsy (age 75 years) showed no foveal cones but a few, scattered cones remaining in the peripheral retina. The side-chains of Ile143 and Leu151 lie in the hydrophobic pocket that stabilizes the conformational structure of EF4 and the replacement by Asn/Thr and Phe, respectively, may interfere with the hydrophobic core and Ca^{2+}-binding loop of EF4 [69].

Functional in vitro analyses of *GCAP1* mutations have been carried out with the Tyr99Cys [85–87], Gly155Gly [88], Ile143AsnThr [84], Glu89Lys, Asp100Glu and Gly159Val [89] substitutions; in all cases, the substitutions alter the level of cyclase inactivation at higher Ca^{2+} concentrations (Fig. 9). Tyr99Cys, Asp100Glu and Asn104Lys lie within EF3 while Ile143AsnThr, Leu151Phe, Glu155Gly and Gly159Val are located in EF4, respectively; in all cases therefore, the number of unaltered Ca^{2+}-binding sites is reduced to two. This is consistent with the findings from in vitro studies that the main functional consequence of these missense mutations is a loss of Ca^{2+} sensitivity, and thereby reduced GC1 inhibition by mutant GCAP1s at higher intracellular Ca^{2+} concentrations. In addition, biochemical analysis showed that the Ile143AsnThr mutant protein adopted a conformation that was more susceptible to proteolysis. The Pro50Leu mutation [90] also gives an altered conformation and an increased susceptibility to proteolysis has been suggested as the disease mechanism in this case.
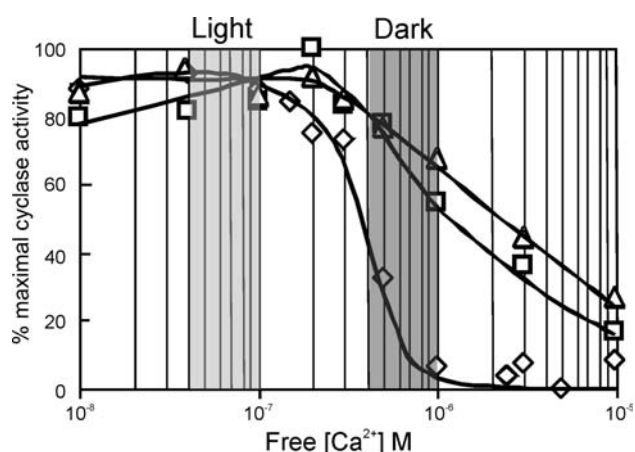

Fig. 9 Ca^{2+} sensitivity of activation of GC1 by wild-type and mutant GCAP1. Activities are presented as percentages of maximum GC1 activation. Shaded boxes indicate Ca^{2+} concentrations in light and dark. WT *diamonds*, Glu155Gly *triangles*, Tyr99Cys *squares*. Data from [88]

Animal models

The double knock-out of *GCAP1* and *GCAP2* in mice has little or no effect on retinal morphology [73]. Extrapolating to humans, this would imply that the loss of functional GCAPs would not cause any overt retinal degeneration and it is perhaps significant that no inherited retinal disorders with null mutations in either *GCAP1* or *GCAP2* have been reported to date. This contrasts with *GC1* where recessive mutations are responsible for LCA. With one exception (Pro50Leu), the dominantly inherited disorders described above reduce the sensitivity of cGMP synthesis to Ca^{2+} via GCAP-activated GCs. The Tyr99Cys disorder has been modelled in the mouse [86] by the expression of a mutant transgene driven by a promoter active in both rods and cones [91]. The enhanced activity of the cyclase in the dark increased cGMP-gated channel activity and elevated the rod outer segment Ca^{2+} concentration. In different lines of transgenic mice, the magnitude of this effect rose in parallel with increases in the expression of mutant GCAP1. Surprisingly, there was little change in the rod photoresponse, indicating that dynamic Ca^{2+}-dependent regulation of cGMP synthesis was preserved. However, the photoreceptors in these mice degenerated, and the rate of the cell loss increased with the level of the transgene expression, unlike in transgenic mice that overexpressed normal GCAP1. The direct role of the elevated cGMP and Ca^{2+} levels arising from the activity of mutant Tyr99Cys and Glu155Gly cyclases in this degeneration is demonstrated, however, by the observation that an increased rate of cGMP removal in the dark achieved via the activation of phosphodiesterase (PDE6) by a constitutively active Gly90Asp rod opsin mutation slows degeneration without affecting the Ca^{2+}-sensitivity of either mutant cyclase [92].

A gene targeting approach introducing a Glu155Gly mutation into the mouse *GCAP1* gene has generated a more accurate model of the disease in man [93]. In these mice, a progressive loss of cone-mediated ERG is evident in both mutant heterozygotes and homozygotes with b-wave amplitudes down to 17% of WT in 12-month old mice. Mutant mice also lack a flicker response to 10- and 15-Hz light stimuli. Rod-mediated b-wave amplitudes were reduced in mutant mice, although in 12 month-old homozygous mice, the rod dominated ERG was still 44% of WT suggesting that the mutation primarily affects cone function. Histological analysis showed significant loss of photoreceptor cells at 5 months of age, with up to 47% loss in homozygotes. Overall therefore, the mutant mice have a phenotype that is consistent with a far greater alteration in cone than rod cell function, in keeping with human disease.

Conclusions

- Both GC1 and GC2 are required for normal photo-transduction. Cone photoreceptors die in the *GC1* knock-out mouse whereas there is no loss of rods. This suggests a more critical role for GC1 in the protection of cones than rods.
- No disease-associated mutations have been reported for *GC2*.
- Recessive mutations in *GC1* are a major cause of LCA in humans. The mechanism of mutant gene action would appear to arise from the loss of functional cyclase.
- Dominant mutations in *GC1* are a major cause of inherited CORD. Mutations are clustered in codon 838 with replacement of Arg with either Cys, Ser or His. The mutant cyclase remains functional but with a reduced sensitivity to Ca^{2+}-dependent inhibition.
- GCAP1 and GCAP2 are both required for normal phototransduction. The pattern of expression differs, with GCAP1 predominantly present in cones and GCAP2 more concentrated in rods.
- In mouse models, the double deletion of *GCAP1* and *GCAP2* does not result in photoreceptor loss. Consistent with this, no recessive disease-associated mutations for either gene have been reported in humans.
- A single dominant missense mutation in *GCAP2* is associated with incomplete penetrance and a spectrum of disease phenotypes that includes RP with and without macular involvement, and macular degeneration.
- Dominant mutations in *GCAP1* result in either cone or CORDs and this may reflect the higher levels of GCAP1 in cones.
- Mutant GCAP1 proteins are functional but generally have a reduced capacity to bind Ca^{2+} as a result of substitutions in the EF hands responsible for Ca^{2+} binding. As a consequence, cyclase activity is less sensitive to inhibition at higher Ca^{2+} concentrations.
- A novel knock-in mouse model of a dominant human *GCAP1* mutation shows significant cone loss with a more limited effect on rod survival and function.

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