## Electronic Supplementary Information for

## Green-fluorescent protein from the bioluminescent jellyfish *Clytia gregaria*: cDNA cloning, expression, and characterization of novel recombinant protein

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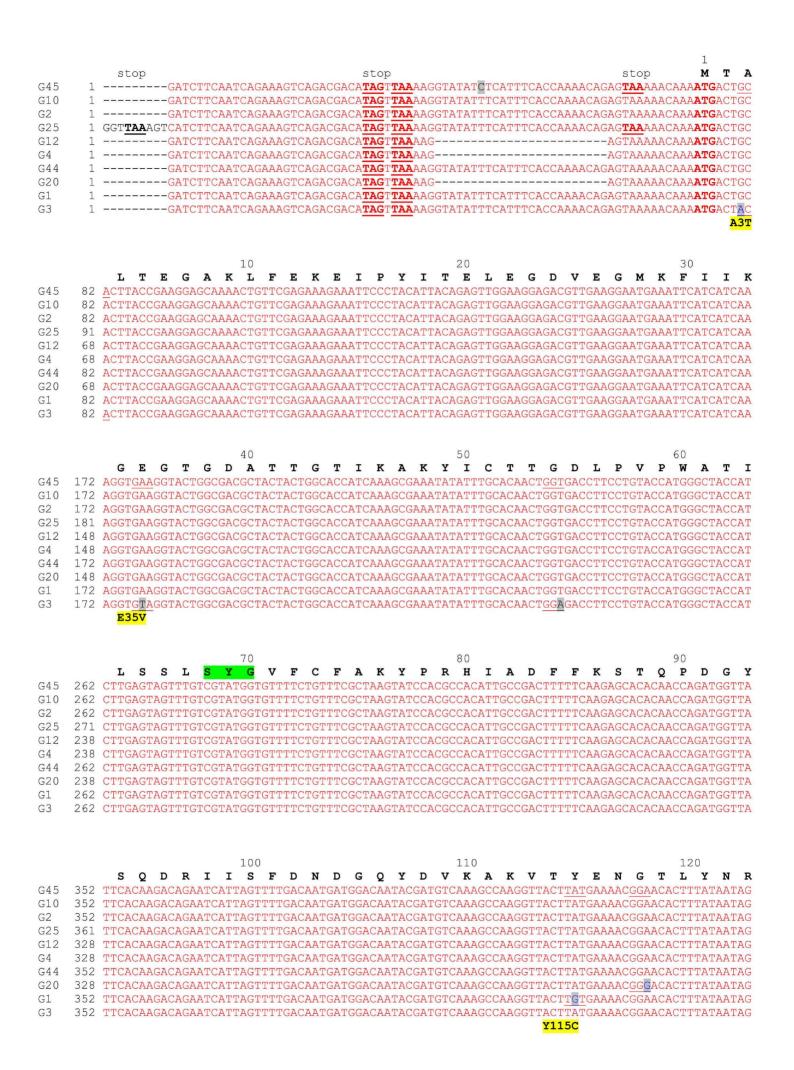
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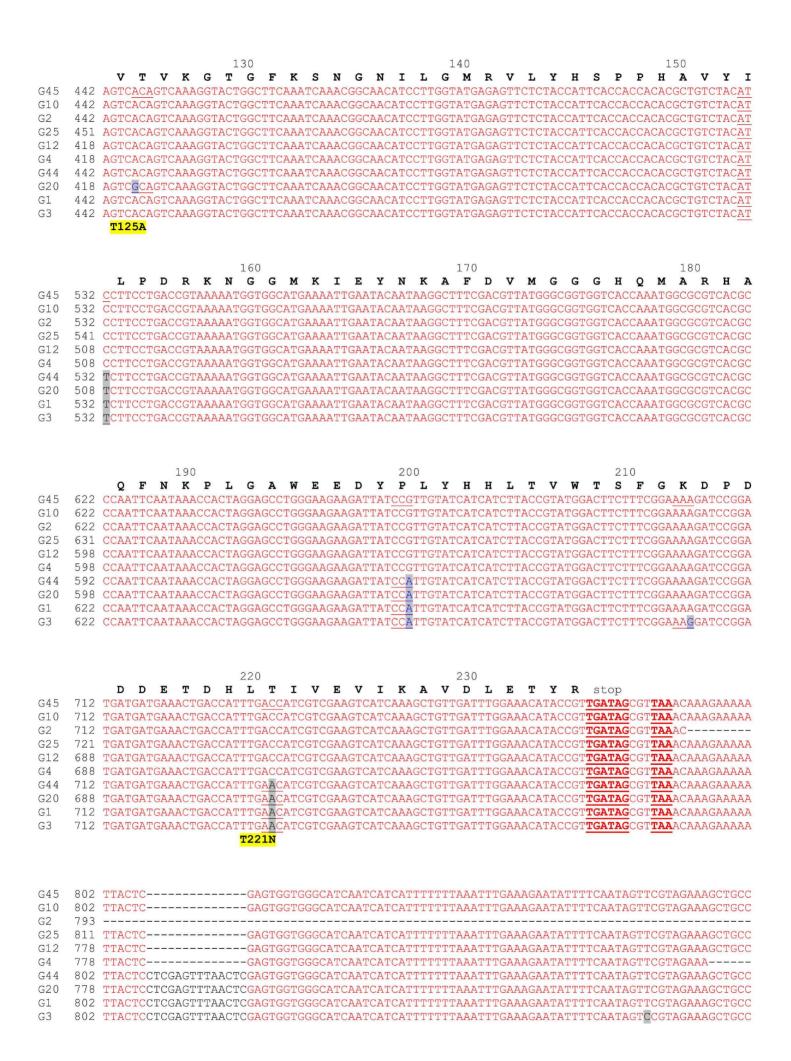
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G45
  G10
  G2.5
G12
  G44
  G1
  892 GTCTATTATAATATTTTATATTTTTGTTTTTATTTCAACCCACTTTTAAAGATCATTCTAAAAGCACTGCTTTTTTCA-----
G3
G45
  968 ATGTATTTATTTACAAATGAATTACTTTGAGTAAGTTTTCTAATCTTCTAAGAAAAAATTACACTTGTTC--------
G10
G2
  977 ATGTATTTACAAATGAATAATTACTTTGAGTAAGTTTTCTAATCTTCTAAGA<mark>AATAAA</mark>ATTACACTTGTTCAAATATCCAAGC-
G25
  944 ATGTATTTACAAATGAATAATTACTTTGAGTAAGTTTTCTAATCTTCTAAGA<mark>AATAAA</mark>ATTACACTTGT<mark>C</mark>C------
G12
  982 ATGTATTTACTACAAATGAATAATTGCTTTGAGTAAGTTTTCTAATCTTCTAAGAAAAATTACACTTGTTCAAATATCCATAAT
G44
  958 ATGTATTTACTTACAAATGAATAATTGCTTTGAGTAAGTTTTCTAATCTTCTAAGAAAAATTACACTTGTCCG-------
G20
  982 ATGTATTTATTTACAAATGAATAATTGCTTTGAGTAAGTTTTCTAATCTTCTAAGA<mark>AATAAA</mark>ATTACACTTGTTCAAATATCCCG---
G3
G45 1045 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
G10 1045 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
  793 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
G25 1066 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
848 AAAAAAAAAAAAAAAAAAAAAAAAAAA
G44 1072 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
  1036 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
G1
  1069 AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
  969 AAAAAAAAAAAAAAAAAAAAAAAAAAA
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Fig. S1. A nucleotide alignment of ten complete cDNA sequences of the cgreGFP genes isolated from one specimen of the jellyfish *Clytia gregaria*: G1, G2, G3, G4, G10, G12, G20, G25, G44 and G45. Start codons (ATG) are marked in bold; stop codons – in bold and underlined. There are four stop-codons in 5-untranslated region of the clone cgreGFP25 before the start codon; other clones have 3 stop-codons in 5-untranslated region. A consensus polyadenylation signal PAS (AAATAA) is indicated by blue box. The amino acid sequence encoded by the cDNAs of the first group (cgreGFP2, 4, 10, 12, 25 and 45) having the same coding part is shown over the alignment. The residues forming the chromophore of cgreGFP are highlighted in green. Nucleotide replacements, making cgreGFP clones (44, 1, 3 and 20) different from the first group of cDNA genes are indicated by grey boxes; the resulting amino acid replacements in cgreGFP44 (T221N), cgreGFP20 (T221N and T125A), cgreGFP1 (T221N and Y115C), and cgreGFP3 (T221N, A3T, and E35V) are shown in yellow boxes under the alignment.

The six isolated cgreGFP cDNAs (cgreGFP2, 4, 10, 12, 25, and 45) are evidently various transcripts of one gene. The heterogeneity of untranslated regions seems to result from an alternative splicing, an imprecise cleavage after polyadenylation signal, and/or from annealing of oligo(dT) primer on any poly(A)-stretch in the 3'-untranslated regions instead of the terminal poly(A) tail.

The cgreGFP clones 44, 1, 3, and 20 differing from each other in 1-2 amino acids have four common nucleotide differences resulting in one common amino acid T221N replacement in coding sequences. These clones initially isolated at library screening were not so bright in comparison with other six cgreGFP clones of the first group. However after multiple passages with selection of bright colonies, these clones became brighter that seems to be the result of additional mutations. Thus, these four cgreGFP cDNAs (44, 1, 3, and 20) might originate from various transcripts of the second GFP gene (allelic gene, for example).

Cl-III	1	MTDTASKYAVKLKTNFEDPK
Cl-I	1	MADTASKYAVKLRPNFDNPK
Cl-II	1	MLWFTNRLLSMSALAARSRLQRTANFHTSILLATDSKYAVKLDPDFANPK
		I
Cl-III	21	WVNRHKFMFNFLDINGNGKITLDE IVSKASDDICAKLGATPAQTQRHQEA
Cl-I	21	WVNRHKFMFNFL <mark>DINGDGKITLDE</mark> IVSKASDDICAKLGATPEQTKRHQDA
Cl-II	51	WINRHKFMFNFLDINGNGKITLDE IVSKASDDICAKLDATPEQTKRHQDA
Cl-III	71	VEAFFKKIGLDYGKEVEFPAFVNGWKELAKHDLKLWSQNKKSLIRNWGEA
Cl-I	71	VEAFFKKIGMDYGKEVEFPAFVDGWKELANYDLKLWSQNKKSLIRDWGEA
Cl-II	101	IEAFFKKMGMDYGKEVPFPEFIKGWEELAKHDLELWSQNKSTLIREWGDA
		II
Cl-III	121	VFDIFDKDGSGSISLDEWKTYGGISGICPSDEDAEKTFKHCDLDNSGKLD
Cl-I	121	VFDIFDKDGSGSISLDEWKAYGRISGICSSDEDAEKTFKHCDLDNSGKLD
Cl-II	151	VFDIFDKDASGSISLDEWKAYGRISGICPSDEDAEKTFKHCDLDNSGKLD
		Identity
Cl-III	17:	1 VDEMTROHLGFWYTLDPNADGLYGNFVP
Cl-I	17:	1 VDEMTRQHLGFWYTLDPNADGLYGNFVP 91.4%
Cl-II	20:	1 VDEMTROHLGFWYTLDPTSDGLYGNFVP 83.8%

Fig. S2. A comparison of the cloned isotype of the Ca<sup>2+</sup>-regulated photoprotein clytin from jellyfish *Clytia gregaria*, clytin-III (Cl-III), with clytin-I (Cl-I, AAA28293) [18] and clytin II (Cl-II, BAG49088) [19] isotypes. Colors mark amino acid similarity according to properties of the side chains. The letters colored by red show identical residues, blue letters represent similar residues, and black letters show nonidentical residues. The Ca<sup>2+</sup>-binding sites I, II, and III are marked in yellow.

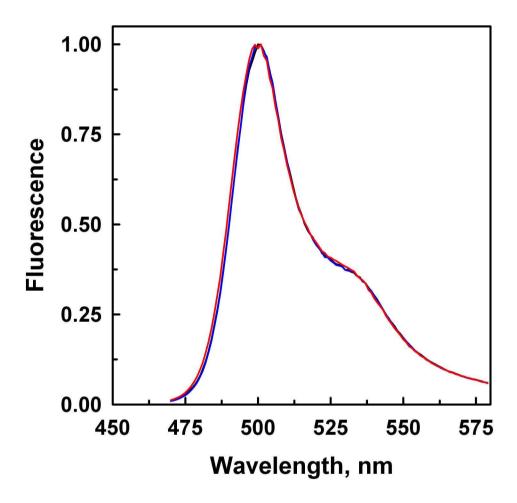


Fig. S3. Normalized fluorescence spectra (excitation at 470 nm) of pure recombinant cgreGFP at pH 5.5 (red), 7.0 (black), and 8.5 (blue).