Assessing the Diversity of Cnidarian Fluorescent Proteins Using Next Generation Sequencing

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FPs & Coral Resilience?

Healthy Coral

Bleached Coral

- 30% increase in ocean acidity since preindustrial times (Hall-Spencer, J.M. et al. 2008)
- 2-3°C increase in SST by 2050 or 2100 (Hoegh-Guldberg, O. et al. 2007)
- 60% mortality expected in the next few decades (Grottoli, A.G et al. 2006)

Fluorescent proteins may help corals survive.
Discovery of FPs

Green Fluorescent Protein first found the jellyfish *Aequorea victoria* (Shimomura, O. 2005)

Shimomura et al. Nobel Prize in Chemistry 2008 (Zimmer, M. 2009)

Widely used in research

Useful as reporter in transgenic cells (Carter et al. 2004)

GFP can be fused to another protein allowing that protein to be visualized in situ.
Fluorescent Proteins

- All FP have similar structure
- Beta-barrel with 11 anti-parallel strands, caped at the ends, to create internal space distinguishable from environment
- Chromophore is inside internal cavity
Fluorescent Colors

- GFP are primary color determinants in corals (Alieva and Konzen 2008)
- Four basic colors - cyan, green, red, and non-fluorescent purple-blue (Alieva and Konzen 2008)

Figure from Technau, Ulrich and Steele, Robert E. (2011)

Fluorescent Colors

- FP expression does not always correlate with the fluorescence color observed (Kao et al. 2007)
Evolution of GFP

- One amino acid substitution can change what color is expressed
- Red FP evolved through several transitions from ancestral GFP (Kao et al. 2007)
- Green FP was derived from a red FP (Alieva et al. 2008)

FP Concentrations Vary By Environment

- Temporal and spatial patterns in FP concentration may exist (Palmer et al. 2009)
- Possible trend of greater FP expression at shallower depths (Kao et al. 2007)
Possible Function

- Wound repair
- Photoprotection
- Enhance photosynthesis
- Visual stimuli

Wound Repair

- Upregulation of FP in injured coral tissues (Palmer et al. 2009)
- Inflammatory-like response - antioxidant activity (Ikmi and Gibson 2010)
Photoprotection

- UV resistance, by converting short wavelengths into longer, less harmful, wavelengths (Dove et al. 2000).
- Coral skeletons convert harmful UV to yellow fluorescence (Kaniewska and Hoegh-Guldberg 2009).
- DNA lesions

Kaniewska and Hoegh-Guldberg 2009

Enhance Photosynthesis

- Symbiotic relationship between corals and zooxanthellae (Kaniewska and Hoegh-Guldberg 2009).
- More efficient photosynthesis than terrestrial plants (Kaniewska and Hoegh-Guldberg 2009).
- Shade zooxanthellae in high light, or enhance light in low light conditions (Dove et al. 2000).
- Longer wavelengths are more efficient for photosynthesis (Dove et al. 2000).
Visual Stimuli

- Warn predators; attract other organisms
- Effects of coloration on reef ecology

Fluorescence in *Nematostella vectensis*

- Bi-fluorescence (Ikmi and Gibson 2010)
- Spatial and temporal variation (Ikmi and Gibson 2010)
- Weak green fluorescence along length of body column, and along lengths of tentacles (Ikmi and Gibson 2010)
- Red fluorescence in oral pole; no red fluorescence in tentacles (Ikmi and Gibson 2010)
It's hard for the audience to keep track of so many different species, so it would have been good to organize them into subsets somehow, e.g., by taxonomic group or by whether the sequences have already been published and why they belong in a specific category (e.g., already validated by others).

**Species with Fluorescent Proteins**

- *Echinopora forskaliana*
- *Discosoma sp.*
- *Entacmaea quadricolor*
- *Nematostella vectensis*
- *Goniopora djiboutensis*
Stylocoenilla sp.

Galaxea fascicularis

Acropora digitifera

Acropora pulchra

Echinophyllia echinata

Conserved motifs in FPs

[Image of a table or chart showing conserved motifs in FPs]
Phylogeny of FPs

Because you make reference to which taxa are related to each other, it would have been useful to provide background on relationships among the major groups of anthozoans, and indicate where your model systems fit.

I really like how you show individual motif arrangements with the species that exhibit them. You might have taken it one step further by showing the sequence alignments of motifs of interest. Also, how do the motifs relate to the structure of FPs that you discussed in the background?

Conserved Motifs

- Acropora pulca
- Galaxea fascicularis
- Acropora hyacinthus
- Acropora millepora
- Echinopora forskaliana
- Goniopora dijiboutiensis
- Montastrea cavernosa
Conserved Motifs

Porites porites
Stylocoeniella sp.
Stylocoeniella sp. 2
Pocillopora damicornis

Conserved Motifs

Echinophyllia echinata
Scleractinia sp.
Platygra lamellina
Missing Motifs

Corallimorph
Corallimorph 2
Edwardsiella lineata
Astrangia lajollaensis

Conclusions

- FPs identified in next-generation sequencing data from 22 species.
- Cannot predict color from sequence or phylogenetic position
- All 23 proteins are clearly related and similar in structure
Future Directions

- “Knock out” fluorescent proteins to determine if
  - UV resistance diminishes
  - Wound healing is compromised
- Investigate how environment (e.g., depth) correlates with FP expression.
- Express “novel” FPs in cell culture to investigate their color.

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References