

SHORT REPORT

Open Access



# Water-soluble chlorophyll-binding proteins from *Arabidopsis thaliana* and *Raphanus sativus* target the endoplasmic reticulum body

Shigekazu Takahashi<sup>†</sup>, Kyoko Aizawa<sup>†</sup>, Katsumi Nakayama and Hiroyuki Satoh<sup>\*</sup>

## Abstract

**Background:** Non-photosynthetic chlorophyll (Chl) proteins called water-soluble Chl-binding proteins are distributed in Brassicaceae plants. *Brassica oleracea* WSCP (BoWSCP) and *Lepidium virginicum* WSCP (LvWSCP) are highly expressed in leaves and stems, while *Arabidopsis thaliana* WSCP (AtWSCP) and *Raphanus sativus* WSCP (RshWSCP) are highly transcribed in floral organs. BoWSCP and LvWSCP exist in the endoplasmic reticulum (ER) body. However, the subcellular localization of AtWSCP and RshWSCP is still unclear. To determine the subcellular localization of these WSCPs, we constructed transgenic plants expressing Venus-fused AtWSCP or RshWSCP.

**Results:** Open reading frames corresponding to full-length AtWSCP and RshWSCP were cloned and ligated between the cauliflower mosaic virus 35S promoter and *Venus*, a gene encoding a yellow fluorescent protein. We introduced the constructs into *A. thaliana* by the floral dip method. We succeeded in constructing a number of transformants expressing Venus-fused chimeric AtWSCP (AtWSCP::Venus) or RshWSCP (RshWSCP::Venus). We detected fluorescence derived from the chimeric proteins using a fluorescence microscope system. In cotyledons, fluorescence derived from AtWSCP::Venus and RshWSCP::Venus was detected in spindle structures. The spindle structures altered their shape to a globular form under blue light excitation. In true leaves, the number of spindle structures was drastically reduced. These observations indicate that the spindle structure was the ER body.

**Conclusions:** AtWSCP and RshWSCP have the potential for ER body targeting like BoWSCP and LvWSCP.

**Keywords:** Chlorophyll, ER body, Water-soluble chlorophyll-binding protein, WSCP

## Background

Chlorophyll (Chl) is a photosynthetic pigment. Most chlorophyll proteins playing a role in photosynthesis are membranous proteins, but highly hydrophilic Chl proteins called water-soluble Chl-binding proteins (WSCPs) have been isolated from various land plants of Chenopodiaceae, Amaranthaceae, Polygonaceae, and Brassicaceae [1]. WSCPs from Chenopodiaceae, Amaranthaceae, and Polygonaceae are photoconvertible, but WSCPs from Brassicaceae do not show this ability [1]. Generally,

photoconvertible and non-photoconvertible WSCPs are called Class I and Class II WSCPs, respectively [1]. Furthermore, Class II WSCPs are categorized into two subclasses, IIA and IIB, based on their Chl *a/b* ratio [1]. To date, *Lepidium virginicum* WSCP (LvWSCP) is the only Class IIB WSCP [1]. Chenopodiaceae WSCPs are members of the domain unknown function 538 superfamily and thus the biological function of these proteins remains unclear [2, 3]. On the other hand, all Class II WSCPs cloned thus far are members of the Kunitz-type trypsin inhibitor family [4–8]. The protease inhibitor activity of Class II WSCPs in young leaves was reported to be important for nitrogen remobilization under stressful conditions [9]. Class II WSCPs are also able to

\*Correspondence: [hsatoh@biomol.sci.toho-u.ac.jp](mailto:hsatoh@biomol.sci.toho-u.ac.jp)

<sup>†</sup>Shigekazu Takahashi and Kyoko Aizawa contributed equally to this work  
Department of Biomolecular Science, Faculty of Science, Toho University,  
2-2-1 Miyama, Funabashi, Chiba 274-8510, Japan

repress reactive oxygen species generation derived from excited Chl [10]. Additionally, *Brassica oleracea* WSCP (BoWSCP) and *Lepidium virginicum* WSCP (LvWSCP) are located in the endoplasmic reticulum (ER) body [6, 8], which contributes to the unique defense system in Brassicaceae [11, 12]. Thus, Class II WSCPs have the potential to act as Chl scavengers during cell disruption to protect healthy cells [6, 8]. Because the molecular structure of Class II WSCPs is quite simple and the complex is quite stable and easy to handle, Class II WSCPs are used as model proteins for characterizing the Chl–Chl and Chl–protein interactions of Chl proteins [13, 14].

BoWSCP and LvWSCP are highly accumulated in leaves and stems [15, 16], while *Arabidopsis thaliana* WSCP (AtWSCP) is expressed in the transmitting tract [5]. Moreover, *Raphanus sativus* var. *raphanistroides* Makino WSCP (RshWSCP) is highly transcribed in floral organs [7]. In contrast to BoWSCP and LvWSCP, the subcellular localization of AtWSCP and RshWSCP is still unclear. Elucidation of the subcellular localization of these proteins will provide clues for understanding the diversity and universality of Class II WSCPs.

## Methods

### Plant materials

*Arabidopsis thaliana* (ecotype, Col-0) was grown on 1 % agar plates containing full-strength MS medium and 1 % sucrose at 22 °C under continuous light. Three-week-old *A. thaliana* was transferred from the plate to Jiffy-7 for further growth.

### Isolation of genomic DNA

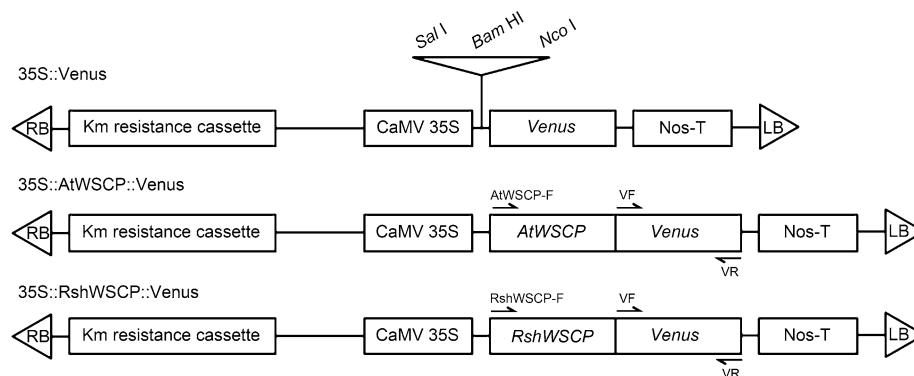
Using a DNeasy mini kit (Qiagen, Venlo, The Netherlands), we extracted and purified genomic DNA from leaves of 2-week-old *A. thaliana* according to the manufacturer's instructions.

### Construction of transgenic *A. thaliana*

The full-length regions of *AtWSCP* and *RshWSCP* were amplified by PCR with specific primer pairs containing a restriction enzyme site: for *AtWSCP*, 5'-GTCGACATGAAGAATCCTTCAGTGATCTCTTTTC-3' and 5'-CCATGGAACCCGGGAAGTATAAGTTGCTAGTAGC-3'; for *RshWSCP*, 5'-GTCGACATGAAGAAACC TTCAGTGACCCCT-3' and 5'-GGATCCGTAGAATGGGAACATCCTCAGACC-3'. Note that we used the *RshWSCP*::pGEM-T easy vector constructed in our previous study for *RshWSCP* amplification [7]. The PCR products were cloned into the pGEM-T easy vector (Promega). After sequence analysis, the correct clones were digested (*SalI* and *NcoI* for the *AtWSCP* construct, *SalI* and *BamHI* for the *RshWSCP* construct) and then the DNA fragments were ligated into the same sites between the cauliflower mosaic virus (CaMV) 35S promoter and *Venus* in a modified pBI101 vector constructed in our previous study. All constructs used to generate transgenic plants are shown in Fig. 1. We introduced the constructs into *A. thaliana* using the floral dip method developed by Clough and Bent [17]. Transgenic plants were selected on 1 % agar plates containing 1 % sucrose, MS medium, 50  $\mu\text{g ml}^{-1}$  kanamycin and 250  $\mu\text{g ml}^{-1}$  cefotaxime sodium salt. Transformation of genes into *A. thaliana* was confirmed by PCR analysis with specific primer pairs (Fig. 1). The sequences of the primers used to detect *Venus* (i.e., VF and VR) and *Actin8* were described in our previous manuscript [8].

### Fluorescence microscopic analysis

To obtain fluorescence images of *A. thaliana* expressing Venus-fused chimeric *AtWSCP* and *RshWSCP* (i.e., *AtWSCP*::*Venus* and *RshWSCP*::*Venus*, respectively), we used an Axioskop2 plus microscope (Carl Zeiss) and Axio Cam (Carl Zeiss) with the Axio Vision software



**Fig. 1** Schematic image of the control and modified Venus constructs for subcellular localization analysis of *Arabidopsis thaliana* WSCP (AtWSCP) and *Raphanus sativus* WSCP (RshWSCP)

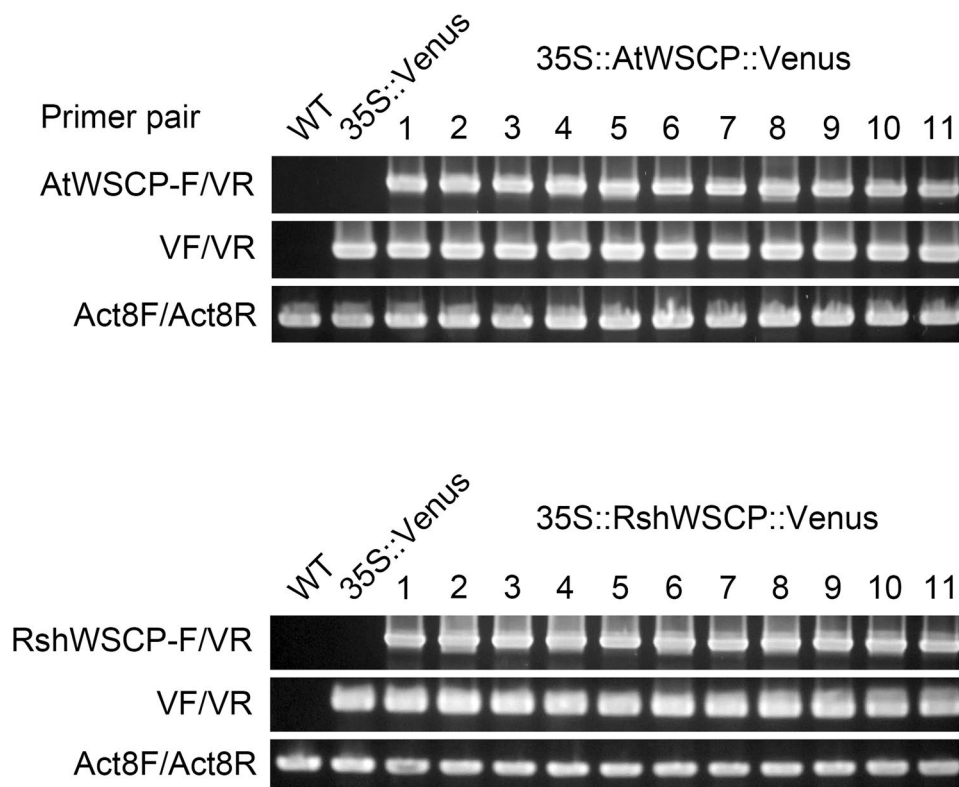
(Carl Zeiss). The T<sub>2</sub> generations of transgenic plants were used for the fluorescence microscopic analysis.

## Results and discussion

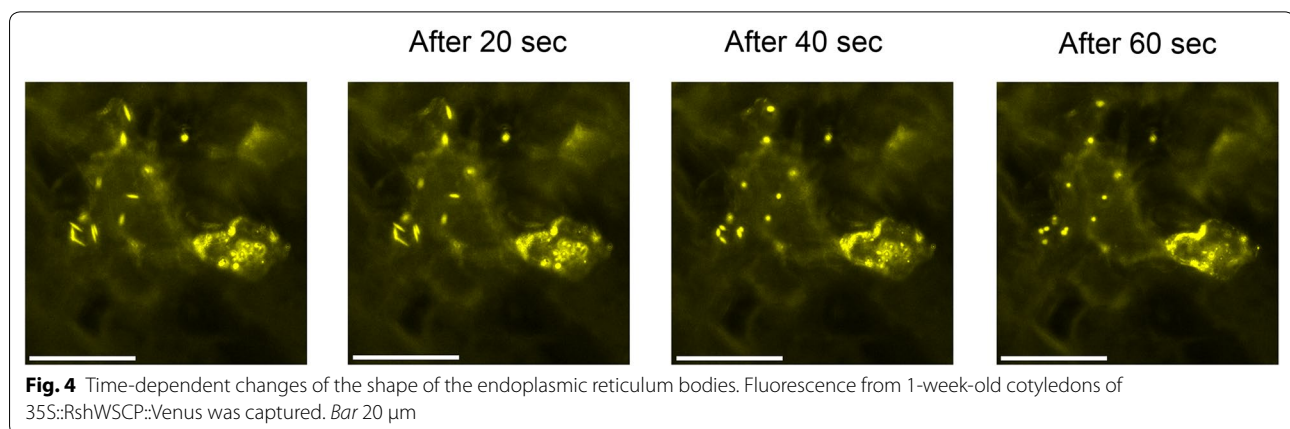
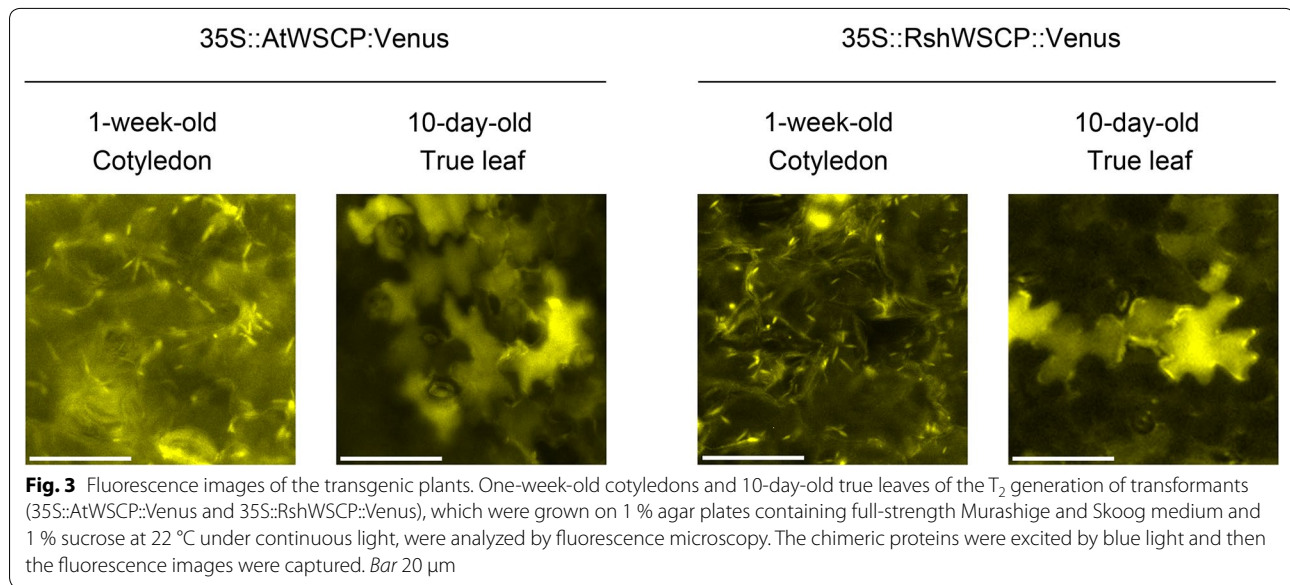
The aim of this study was to reveal the subcellular localization of AtWSCP and RshWSCP. In previous studies, we characterized the subcellular localization of BoWSCP and LvWSCP by analyzing transgenic *A. thaliana* expressing Venus-fused chimeric BoWSCP or LvWSCP (i.e., BoWSCP::Venus or LvWSCP::Venus) and found that both chimeric proteins were located in ER bodies [6, 8]. Here, we constructed transgenic AtWSCP::Venus or RshWSCP::Venus. Similar to BoWSCP and LvWSCP, both AtWSCP and RshWSCP possess an N-terminal extension, which is predicted to be a signal peptide. Thus, we introduced Venus at the C-termini of AtWSCP and RshWSCP. All constructs used to generate transgenic *A. thaliana* are shown in Fig. 1. The correctness of the transformants (35S::AtWSCP::Venus and 35S::RshWSCP::Venus) was confirmed by PCR analysis with the primer sets AtWSCP-F or RshWSCP-F/VR and VF/VR (Fig. 1). As shown in Fig. 2, we obtained a number of transformant lines.

Figure 3 shows fluorescence images of leaves at different developmental stages from the transformants. In 1-week-old cotyledons of 35S::AtWSCP::Venus and 35S::RshWSCP::Venus plants, the fluorescence from Venus was detected in spindle-shaped structures. In the true leaves, however, most of these structures had disappeared and Venus fluorescence was instead detected in the vacuole. Furthermore, the spindle-shaped structures changed shape from spindle to globular during the fluorescence microscopic analysis with the high irradiation of blue light (Fig. 4). Brassicaceae plants have a unique spindle-shaped organelle called the ER body [11]. It was reported that the ER body changes its structure to a globular form and then fuses with the vacuole under high salinity conditions [18]. Recently, Gotté et al. reported that *Raphanus sativus* also has the ER body and that its shape was changed by methyl jasmonate treatment [19]. We therefore concluded that AtWSCP and RshWSCP can potentially target the ER body and the vacuole like BoWSCP and LvWSCP, but they do not target chloroplasts.

Because AtWSCP has protease inhibitor activity [20] and accumulates in the transmitting tract in the



**Fig. 2** PCR analysis to confirm the correct insertion of cauliflower mosaic virus 35S promoter (35S)::AtWSCP::Venus and 35S::RshWSCP::Venus into *A. thaliana*. Primer sites (AtWSCP-F, RshWSCP-F, VF, and VR) are shown in Fig. 1. *Actin8* was used as a control



gynoecium and silique [5], Bechtas et al. hypothesized that the protease inhibitor activity of AtWSCP might be important for the formation of the transmitting tract. Note that we could not find any difference between the transformants and wild type *A. thaliana*. Further analysis of the AtWSCP null-mutant will provide clues to elucidate the biological function of AtWSCP.

### Conclusion

To our knowledge, this is the first report describing the subcellular localization of floral organ-expressed WSCPs (i.e., AtWSCP and RshWSCP). Similar to leaf-expressed WSCPs (i.e., BoWSCP and LvWSCP), AtWSCP and RshWSCP target the ER body.

### Abbreviations

Chl: chlorophyll; ER: endoplasmic reticulum; WSCP: water-soluble chlorophyll-binding proteins.

### Authors' contributions

ST designed the research. ST and KA performed the experiments. ST and HS wrote the paper. NK and HS supervised the work. All authors read and approved the final manuscript.

### Compliance with ethical guidelines

### Competing interests

The authors declare that they have no competing interests.

Received: 21 January 2015 Accepted: 12 August 2015

Published online: 20 August 2015

### References

1. Satoh H, Uchida A, Nakayama K, Okada M. Water-soluble chlorophyll protein in Brassicaceae plants is a stress-induced chlorophyll-binding protein. *Plant Cell Physiol.* 2001;42:906–11.
2. Takahashi S, Yoshikawa M, Kamada A, Ohtsuki T, Uchida A, Nakayama K, Satoh H. The photoconvertible water-soluble chlorophyll-binding protein of *Chenopodium album* is a member of DUF538, a superfamily that distributes in Embryophyta. *J Plant Physiol.* 2013;2013(170):1549–52.

3. Takahashi S, Abe E, Nakayama K, Satoh H. Identification of genes encoding photoconvertible (Class I) water-soluble chlorophyll-binding proteins from *Chenopodium ficifolium*. *Biosci Biotechnol Biochem*. 2015 (in press). doi:10.1080/09168451.2014.972326.
4. Satoh H, Nakayama K, Okada M. Molecular cloning and functional expression of a water-soluble chlorophyll protein, a putative carrier of chlorophyll molecules in cauliflower. *J Biol Chem*. 1998;273:30568–75.
5. Bektas I, Fellenferg C, Paulsen H. Water-soluble chlorophyll protein (WSCP) of *Arabidopsis* is expressed in the gynoecium and developing silique. *Planta*. 2012;236:251–9.
6. Takahashi S, Yanai H, Nakamaru Y, Uchida A, Nakayama K, Satoh H. Molecular cloning, characterization and analysis of the intracellular localization of a water-soluble chlorophyll-binding protein from Brussels sprouts (*Brassica oleracea* var. *gemmifera*). *Plant Cell Physiol*. 2012;53:879–91.
7. Takahashi S, Ono M, Uchida A, Nakayama K, Satoh H. Molecular cloning and functional expression of a water-soluble chlorophyll-binding protein from Japanese wild radish. *J Plant Physiol*. 2013;170:406–12.
8. Takahashi S, Yanai H, Oka-Takayama Y, Zanma-Sohtome A, Fujiyama K, Uchida A, Nakayama K, Satoh H. Molecular cloning, characterization and analysis of the intracellular localization of a water-soluble chlorophyll-binding protein (WSCP) from Virginia pepperweed (*Lepidium virginicum*), a unique WSCP that preferentially binds chlorophyll *b* in vitro. *Planta*. 2013;238:1065–80.
9. Desclos M, Dubousslet L, Etienne P, Le Caherec F, Satoh H, Bonnefoy J, Ourry A, Avice JC. A proteomic profiling approach to reveal a novel role of *Brassica napus* drought 22 kD/water-soluble chlorophyll-binding protein in young leaves during nitrogen remobilization induced by stressful conditions. *Plant Physiol*. 2008;147:1830–44.
10. Schmidt K, Fufezan C, Krieger-Liszkay A, Satoh H, Paulsen H. Recombinant water-soluble chlorophyll protein from *Brassica oleracea* var. *Botrys* binds various chlorophyll derivatives. *Biochemistry*. 2003;42:7427–33.
11. Yamada K, Hara-Nishimura I, Nishimura M. Unique defense strategy by the endoplasmic reticulum body in plants. *Plant Cell Physiol*. 2011;52:2039–49.
12. Yamada K, Nagano AJ, Nishina M, Hara-Nishimura I, Nishimura M. Identification of two novel endoplasmic reticulum body-specific integral membrane proteins. *Plant Physiol*. 2013;161:108–20.
13. Renger G, Pieper J, Theiss C, Trostmann I, Paulsen H, Renger T, Eichler HJ, Schmitt FJ. Water soluble chlorophyll binding protein of higher plants: a most suitable model system for basic analyses of pigment-pigment and pigment-protein interactions in chlorophyll protein complexes. *J Plant Physiol*. 2011;168:1462–72.
14. Alster J, Lokstein H, Dostal J, Uchida A, Zigmantas D. 2D spectroscopy study of water-soluble chlorophyll-binding protein from *Lepidium virginicum*. *J Phys Chem B*. 2014;118:3524–31.
15. Murata T, Murata N. Water-soluble chlorophyll-proteins from *Brassica nigra* and *Lepidium virginicum*. *Carnegie Inst Wash Yearb*. 1971;70:504–7.
16. Kamimura Y, Mori T, Yamasaki T, Katoh S. Isolation, properties and a possible function of a water-soluble chlorophyll *a/b*-protein from Brussels sprouts. *Plant Cell Physiol*. 1997;38:133–8.
17. Clough SJ, Bent AF. Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J*. 1998;16:736–43.
18. Hayashi Y, Yamada K, Shimada T, Matsushima R, Nishizawa N, Nishimura M, Hara-Nishimura I. A proteinase-storing body that prepares for cell death or stresses in the epidermal cells of *Arabidopsis*. *Plant Cell Physiol*. 2001;42:894–9.
19. Gotté M, Ghosh R, Bernard A, Nguema-Ona E, Vicié-Gibouin M, Hara-Nishimura I, Driouch A. Methyl jasmonate affects morphology, number and activity of endoplasmic reticulum bodies in *Raphanus sativus* root cells. *Plant Cell Physiol*. 2015 (in press). doi:10.1093/pcp/pcu141.
20. Halls CE, Rogers SW, Oufattole M, Ostergard O, Sevansson B, Rogers JC. A Kunitz-type cysteine protease inhibitor from cauliflower and *Arabidopsis*. *Plant Sci*. 2006;170:1102–10.

**Submit your next manuscript to BioMed Central and take full advantage of:**

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at  
[www.biomedcentral.com/submit](http://www.biomedcentral.com/submit)

