

Some Like It Hot

Switching Biology on the Microscope

The combination of advanced molecular biology and multi-dimensional microscopy can generate fascinating new insights into key biological functions and processes. Here we report on the development of a new method and device to investigate temperature sensitive mutations and/or temperature dependent biological processes at unprecedented high temporal-spatial resolution.

Studying Gene Functions

Forward and reverse genetics methods are powerful tools to study gene products and their functions. However, if a protein participates in several different processes, the terminal phenotype will be a representation of cumulative rather than specific defects. To find out about the relevance of a gene for a specific process or the exact point at which the gene's activity is required, it is necessary to investigate the *in vivo* function of a gene product in a temporally and spatially controlled manner.

Monitoring at High Temporal and Spatial Resolution

In order to be able to address these questions, scientists have generated different

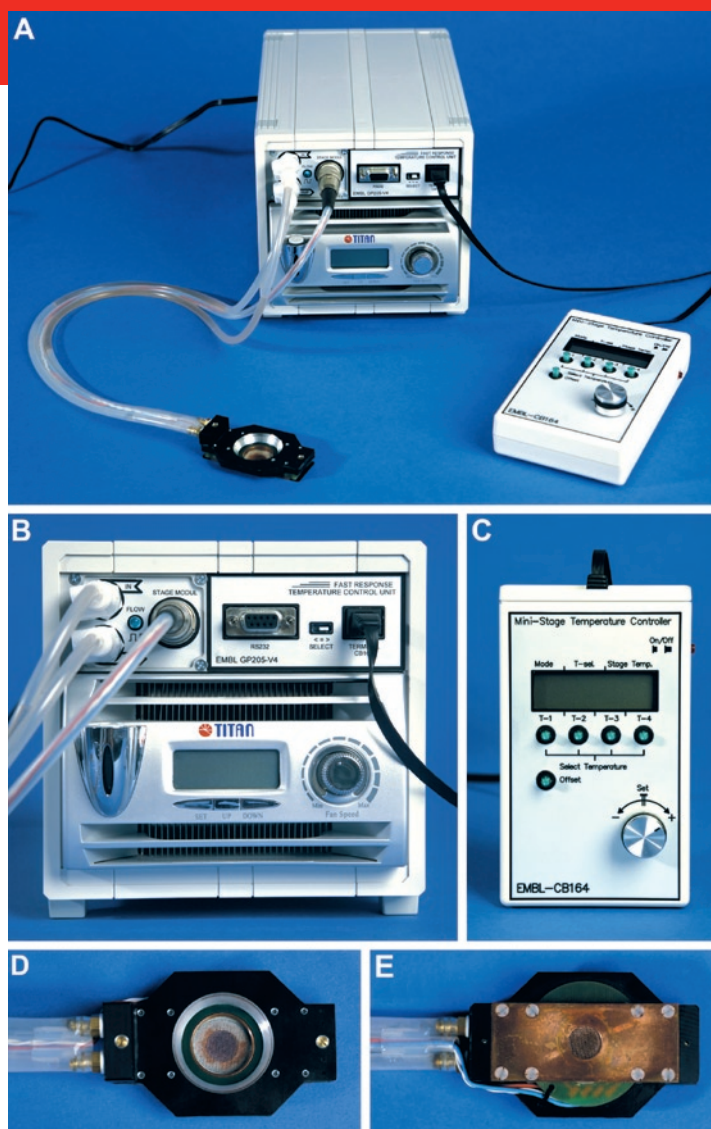


Fig. 1: (A) The newly developed fast response temperature controller, with enlargements of (B) the unit's frontal part, (C) mini stage temperature controller, (D) top and (E) bottom of heating-cooling copper plate. Specimens were mounted on 2% agarose between two coverslips, which were glued to the copper plate (E) with vacuum grease and placed into the slide holder of Leica confocal microscope AOB5 SP2.

cold- and heat-sensitive gene mutations in a large variety of species. The advantage of such mutations is that at the permissive temperature the mutation is silent and the mutant phenotype manifests only at restrictive temperature. The fact that some of these mutations respond rapidly to temperature shift raise the possibility to monitor their effects at high temporal resolution. To be able to also monitor these effects at high spatial resolution required the development of a fast acting temperature controller compatible with high resolution microscopy.

Temperature Sensitive *baf-1* mutation

When scientists at the European Molecular Biology Laboratory (EMBL, Heidelberg) and at the Institute for Research in Biomedicine (Barcelona) analyzed a temperature sensitive mutation in a *Caenorhabditis elegans* gene called *baf-1*, they determined that *baf-1* is required for nuclear envelope (NE) formation at the end of mitosis [1]. The scientists found that at permissive temperature, the mutant protein was fully functional and it was properly phosphorylated, while at restrictive temperature it was inactive and was not phosphorylated. To be able to specifically inactivate this protein at different time points of mitosis before and after the NE reformation, a fast response temperature controller was developed at the electronic workshops at EMBL and tested by the scientists at EMBL and the EMBL Advanced Light Microscopy Facility (fig. 1). Relying on Peltier technology, the novel device could be used for recordings at constant temperature or for shifting between 4 and 38 °C at rate of ~ 1 °C/sec with ±0.3 °C long term temperature accuracy (for more information see [2]). At the same time, software that could compensate for the shift in focus resulting from the temperature change was developed (Timo Zimmermann, pers. comm.).

Microscopy of Mutant *C. elegans* Embryos

Utilizing this device in combination with simultaneous transmission and epi-fluorescence confocal time lapse microscopy, mutant *C. elegans* embryos were recorded at permissive temperature and shifted to restrictive temperature at dif-

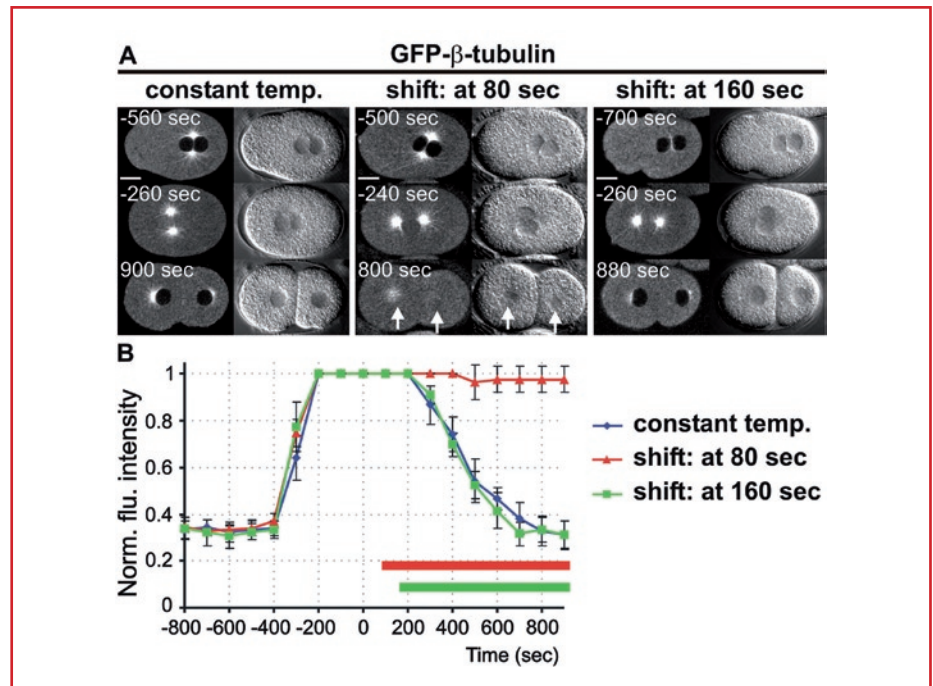


Fig. 2: Temperature sensitive *baf-1* mutant *C. elegans* embryos recorded using the fast response temperature controller. All the embryos were recorded from the pronuclear stage through the first mitotic division until the two cell stage. Time is relative to anaphase onset (0 sec).

(A) Temperature sensitive *baf-1* mutant embryo grown and recorded at constant permissive temperature behaves like wild type embryos. The NE is properly formed and therefore during interphase it excludes cytoplasmic molecules above 45 kDa. Thus soluble GFP-β-tubulin is also excluded from the nuclear space (left panel). Temperature sensitive *baf-1* mutant embryo grown and recorded at permissive temperature and shifted to the restrictive before NE reformation, 80 sec after metaphase-to-anaphase transition (middle panel). Note that this temperature shift completely disrupts the formation of a functional NE and that the soluble GFP-β-tubulin is not excluded from the nuclear space. A similar temperature shift has no effect on wild type embryos (data not shown). Temperature sensitive *baf-1* mutant embryo grown and recorded at permissive temperature and shifted to the restrictive temperature after NE reformation, 160 sec after the metaphase-to-anaphase transition (right panel). Note that inactivation of the *baf-1* protein function has no effect on already assembled NEs. Bars, 10 μm.

(B) Nuclear exclusion was quantified from time-lapse recordings by dividing nuclear fluorescence intensity by cytoplasmic fluorescence intensity after background subtraction. Time is relative to anaphase onset (0 sec). For each condition, 3–4 embryos were analyzed.

ferent time points with respect to the onset of mitosis. With a series of such temperature shift experiments, the scientists could show that *baf-1* is specifically involved in NE formation and that the observed NE defect at restrictive temperature is not a secondary consequence of upstream defects (fig. 2) [1].

As there are a multitude of such temperature sensitive alleles in a number of organisms, it is to be expected that the methods used in this study will also help other scientists to more exactly define the functions of genes required for a variety of different biological processes in a way that was previously either difficult or impossible.

Acknowledgement

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References

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