

Investigation of the sub-cellular localization of Rqh1 in fission yeast

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Background and Purpose

rqh1^{+} is the only member of the RecQ helicase family found in *Schizosaccharomyces pombe*. There are other homologues of RecQ helicases found in bacteria, yeasts, flies, frogs and humans. Members of this DNA helicase family are interesting because they are thought to be involved in maintaining genomic integrity. Mutation of members of this family in humans is responsible for Werner's syndrome, Bloom's syndrome and Rothmund-Thomson syndrome. Patients with these diseases show a predisposition to cancer or premature ageing, and fission yeast cells with a defective *rqh1*$^{+}$ gene show reduced viability and abnormal chromosomal loss even under normal growth condition.

To gain further insight into the role(s) of Rqh1 in *S. pombe*, the sub-cellular localization has been investigated using indirect immunofluorescence. Rqh1 is thought to be localized in the nucleus especially in nucleolar foci. However this is not completely clear because of the difficulty of seeing the nucleolus. Therefore nucleolar recognition was attempted using two different methods to see where Rqh1 is localizing.

Before doing immunofluorescence, it was necessary to purify a lot of anti-Rqh1 antibody, N156ECS. First purification of the antibody was carried out using a modified Western blotting method, called Western Method. However, this resulted in a lot of dilute antibody which had to be concentrated x40 for immunofluorescence. For the successful immunofluorescence, a rapid and high efficiency purification method was constructed. It will be useful not only for the immunofluorescence study but also for the other research into *rqh1*$^{+}$ mechanisms.

Materials and Methods

The indirect immunofluorescence was done using *Schizosaccharomyces pombe*, normal WT and *rqh1* Δ strains or WT and *rqh1* Δ *leu*$^{-}$ strains for transformation. The anti-Rqh1 antibody, N156ECS, used in immunofluorescence was purified from rabbit serum by using Western Method or Affi-Gel15 Method. Affi-Gel15 Method was constructed using Affi-Gel15 agarose beads support obtained from QIAGEN.

Result and Discussion

The new antibody purification method, the Affi-Gel15 Method, was successfully constructed. N156ECS purified by this method worked on Western blotting and immunofluorescence and was x20- x40 more concentrated than that purified using the Western Method. This method is also very useful as there is no need to re-couple more antigen to beads to purify more N156ECS once antigen is coupled to beads.

The plasmid containing GFP which localizes to the nucleolus was transformed into yeast cells and cells showing good fluorescence were obtained. Fixation conditions were revised for the use of these transformed cells. Growing in YE5S medium instead of EMM Ahu medium is necessary to fix the GFP transformed strains properly to use for immunofluorescence. Rqh1 foci in normal WT cells were seen in the nucleolus. And also in GFP transformed WT cells, Rqh1 looked like it was localizing to the nucleolus but further experiments were required to confirm this.

Future work

Immunofluorescence examination using GFP transformed cells should be done to confirm the localization of Rqh1 in the nucleolus. In addition, using the delta-vision microscope is recommended to see the fluorescence in more detail. It will then become possible to say exactly where Rqh1 is localizing. Also it would be interesting to see whether the localization of Rqh1 changes during each cell cycle or after DNA damage.

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