Identification of novel homologue genes for DNA repair enzyme AP endonuclease and analysis of their function

DNA 修復酵素 AP エンドヌクレアーゼの新規ホモログ遺伝子の同定とその機能解析

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Summary

Backgrounds

DNA is constantly damaged in cells, and accumulation of damaged DNA causes serious consequences, such as aging and cancer. In addition to AP endonuclease activity, AP endonuclease also exhibits 3′-phosphodiesterase activity and/or 3′-5′ exonuclease activity. AP endonuclease activity and 3′-phosphodiesterase activity are necessary for base excision repair. On the other hand, it is unclear how the 3′-5′ exonuclease activity of the AP endonuclease functions in the cell. And there are several subtypes of AP endonuclease. In mammalian cells, APEX1 type AP endonuclease shows strong AP endonuclease activity and function in base excision repair as a major AP endonuclease. In contrast, the cellular functions of other type of AP endonuclease are not clear. In the present study, I focused on P0, which is also function as a ribosomal protein, and APEX2 type AP endonucleases to investigate their function in cells.

Methods

Candidate AP endonucleases of *Ciona intestinalis* were searched by BLAST using amino acid sequences of AP endonuclease previously reported. Detection and substrate characterization of enzymatic activities were conducted by using oligonucleotide and purified proteins. The complementation assay was performed by using AP endonuclease-

deficient *Escherichia coli*. In the study using *Saccharomyces cerevisiae,* the cellular effects of APEX2 type AP endonuclease *APN2* deficiency were investigated. Functional analysis was performed under nitrogen starvation. Mitochondria quantity was measured by a confocal microscope using a mitochondria specific fluorescent dye, mitotracker. Colony size was measured by the image analysis using the photographs of plates obtained by the colony formation assay. Then, the small colony ratio was calculated among total colony number. The time dependent change in survival rate under nitrogen starvation was conducted by colony formation assay using *APN1*, which is another AP endonuclease gene in *S. cerevisiae*, and *APN2* deficient mutants. An experiment using ρ0 strain, which has nonactive mitochondria was performed to examine the possibility that mitochondrial abnormality affects the cell survival. Methoxyamine susceptibility was investigated by the colony formation assay.

Results and Discussion

I started with a study on AP endonuclease in *Ciona intestinalis*. In *C. intestinalis,* only APEX1 type AP endonuclease (CiApex1) has been identified. Then I identified the new candidate AP endonucleases in *C. intestinalis*. From the BLAST search, I identified the candidate AP endonuclease genes in *C. intestinalis* for APEX2 type *CiApex2* and P0 type *CiP0* and conducted biochemical analysis using purified proteins. As a result, these two candidate proteins did not show both the AP endonuclease activity and the 3'-phosphodiesterase activity. On the other hand, both proteins showed 3'-5' exonuclease activity. Characterization of 3'-5' exonuclease activity's substrate suggested that both proteins are involved in the induction of DNA damage response. Furthermore, from the complementation assay, both proteins complement the oxidative stress sensitivity of AP

endonuclease deficient *Escherichia coli*. From these results, it is suggested that unlike the APEX1 type AP endonuclease, 3'-5' exonuclease activity of Apex2 and P0 type AP endonuclease contribute to the oxidative stress resistance. Then I investigated the cellular effects of APEX2 type AP endonuclease (Apn2) deficiency using *S. cerevisiae*. Nitrogen starvation is known to induce extensive degradation of intracellular macromolecules. Under nitrogen starvation, mitochondria were gradually reduced by degradation, but the amount of mitochondria was higher in the *APN2* deficient strain compared with wild type strain. When culturing under nitrogen starvation, *APN2* deficient strain increased small colony ratio, which is the characteristic of yeast strain with inactivated mitochondria. These results suggested that Apn2 contribute to maintain the mitochondrial quality by decreasing the harmful mitochondria generated under the nitrogen starvation. From the experiment focused on cell survival, it is found that the survival rate of wild type and *APN1* deficient strains were gradually decreased by long-term culture under nitrogen starvation, whereas *APN2* deficient strain maintained the cell survival. This result indicates that Apn2 regulate cell survival under the nitrogen starvation by the mechanism different from the way of Apn1. Moreover, *APN2* deficient strain with inactivated mitochondria also did not decrease the survival rate during the long-term cultivation under nitrogen starvation. This result indicates that the Apn2 function in cell survival is independent from the Apn2 function in mitochondrial quality maintenance. Furthermore, from the result of drug susceptibility test under nitrogen starvation, it was found that *APN2* deficiency decrease the cell death induction caused by AP site repair inhibition. This result indicates that AP sites are related to the cause for the decrease of cell death induction under nitrogen starvation in *APN2* deficient strain.

Conclusion

The present study shows that APEX2 or P0 type AP endonucleases maintains the mitochondrial quality and contributes to the cell growth and survival under stressed condition through the mechanisms different from those of APEX1.