

澳洲球型海綿細胞色素氧化酶
次單位 I 基因介入子之自我剪切能力研究

詹蕙鎂*, 徐基新

後生動物中僅少數的粒線體基因含有介入子，介入子在 RNA 的合成過程中，須經切除才能使此段基因合成具功能性的成熟型 RNA，此介入子則自己形成 lariat 構型；在各類真核生物的基因中又可因 RNA 不同的剪切方式分為具自我剪切能力的第一型介入子、第二介入子，還有剪接體介入子和轉運 RNA 介入子。

本篇研究在澳洲球型海綿粒線體的細胞色素氧化酶次單位 I 基因中，發現具有第一型介入子特徵的序列，經分析可得知其中具有兩段 LAGLI-DADG 內切酶，可使細胞色素氧化酶次單位 I 基因介入子轉置到其他生物或本身的同源基因位置。從海綿的 DNA 和 RNA 序列比較可得知此段基因的確有經剪切的情形，且在 RNA 序列中缺少的正是整段完整的介入子序列；再將整段介入子轉型至大腸桿菌中，同樣可看到介入子經剪切的現象。另外，在本次實驗的海綿本身和轉型的大腸桿菌中，皆可經由 RT-PCR 方式觀察得到構成 lariat 的介入子。結果顯示，不論是在真核的海綿或原核的大腸桿菌表現系統中，細胞色素氧化酶次單位 I 基因介入子皆可從其經剪切並構成 lariat 的現象，證實具自我剪切的功能。

關鍵字：澳洲球型海綿，自我剪切，第一型介入子，LAGLIDADG 內切酶

Self-splicing in the cytochrome oxidase I gene of *Cinachyrella australiensis*

Hui-Mei Chan* and Chi-Shin Hsu

Institute of Marine Biotechnology and Resources, National Sun Yat-sen University

Most metazoan mitochondrial genomes lack introns. Those genes with introns must undergo a cleavage reaction to produce the mature, functional form of RNA, and then introns form lariat. In all kinds of eukaryote, four major categories of splicing can be divided into Group I, Group II, spliceosomal, and nuclear tRNA introns. On their secondary structure, either Group I or Group II are also called self-splicing introns.

Here the putative intron in the cytochrome oxidase I gene (*cox1*) of *Cinachyrella australiensis* has Group I intron characteristics and encodes two putative homing endonucleases with the amino acid motif of the LAGLIDADG family which perform intron translocation function. Self-splicing in the *cox1* of sponge was demonstrated by comparing with the length of DNA and RNA sequence. Indeed, it lacks a whole intron at the RNA level. Besides, we transform the entire intron into *E.coli*. Similarly, we also can observe the self-splicing of *E.coli* system. On the other method for checking self-splicing *cox1* intron was processed by RT-PCR to get the spliced intron. In this experiment, the intron with lariat form would be detected either from sponge or *E.coli* system. In conclusion, self-splicing in *cox1* intron was worked out for gene with splicing-ligation reaction and intron cyclization whether on eukaryotes *Cinachyrella australiensis* or on prokaryotes *E.coli* system.

Keywords: *Cinachyrella australiensis*, self-splicing, Group I intron, LAGLIDADG endonuclease