# 薬学博士池原森男氏及び薬学博士大塚榮子氏の「核酸の合成と機能に関する研究—合成 *ras* 遺伝子関連の研究を中心として」（共同研究）に対する授賞審査要旨

## I. RNA 合成法の開発と tRNA 全合成の研究

池原氏らは一九六五年プリン-8-シクロヌクレオシドを最初に合成し、以来ヌクレオシド糖部変換反応に新ルートを開き、初めてリボヌクレオシドであるアデノシンから2′-デオキシアデノシンへの化学的誘導に成功した。これらの合成において開発されたプリンヌクレオシドの高収率な8-ブロム化および8-ヒドロキシ化反応を用いて大塚氏らはDNA中に生ずる代表的損傷である7、8-ジヒドロ-8-オキソデオキシグアノシンの合成を行い、がん遺伝子 *ras* における変異の機構の一つを明らかにした。

池原、大塚両氏は一九六七年以来、転移RNA（tRNA）のヌクレオチド配列が明らかにされるのと平行してその合成に取り組み、各種の保護基の研究を行った。RNAブロック合成のためには、末端リン酸エステルの保護基のみを中性条件下除去をする必要のあることから、中性付近で亜硝酸イソアミル処理によって除去可能な芳香族アミデート基を用いる方法を開発した（図1）。次にRNAに特有の2′水酸基の保護基として、光照射により除去可能なオルトニトロベンジル基を導入した（図2）。これらの方法を駆使して十一個のRNA断片を合成し、酵素的結合反応を

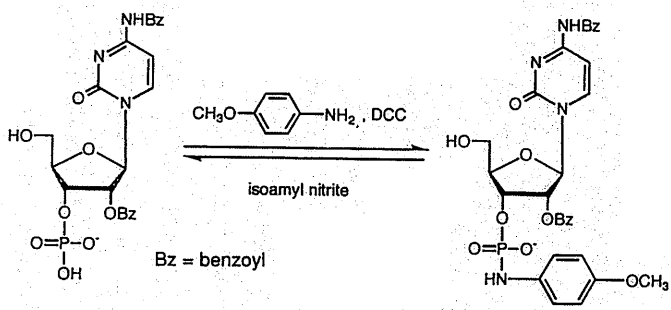
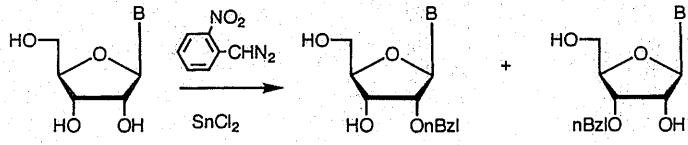



図1　芳香族アミデートによるリン酸エステルの保護




N-benzoyladenosine, B= N-benzoyladenin-9-yl (bzA)
N-benzoylcytidine, B= N-benzoylcytosin-1-yl (bzC)
N-isobutyrylguanosine, B= N-isobutyrylguanin-9-yl (ibG)
Uridine, B= uracil-1-yl (U)

nBzl = *o*-nitrobenzyl

図2　2′(3′)-オルトニトロベンジルヌクレオシドの合成

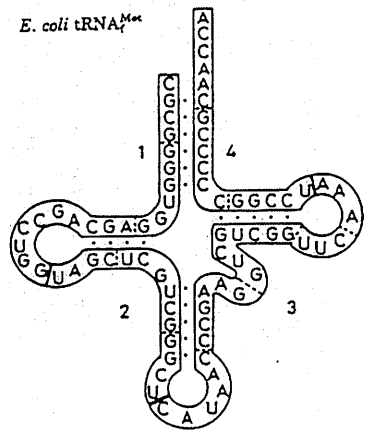



図3　ホルミルメチオニン tRNA

用いることにより、一九七九年蛋白生合成の開始に関与するホルミルメチオニンtRNAの全合成を達成した（図3）。このようなtRNA全合成により、その分子の部分的変換も容易となり、アミノアシル化酵素などによって認識される機能部位を明らかにした。

Ⅱ．DNAの新合成法と遺伝子合成

次に、RNA合成のために開発した芳香族リン酸アミデート法をDNAの合成にも適用し、リプレッサー結合部位オペレーター二本鎖DNAの大量合成を行い、その構造研究を行った。また、未知のアミノ酸コドンを持つメッセンジャーRNAと塩基対を形成させるDNAとして、デオキシイノシンを含むDNA断片を合成し、これを用いることによりそれまで困難であったコレシストキニン遺伝子などの塩基配列決定を可能とした。

以上の結果をふまえ、両氏は各種の蛋白質遺伝子の合成を行い、ヒト成長ホルモン遺伝子をはじめ、がん遺伝子ras、RNaseT1、ヒトリゾチーム、RNaseH、T4-エンドヌクレアーゼVおよびその変異体遺伝子を合成し、その大量発現によって、それらの蛋白質を結晶に導き、X線回析像を分析して三次元構造を明らかにした。就中RAS蛋白質は初めての発がん遺伝子産物の三次元構造として注目された。

Ⅲ．リボザイムの合成と機能の研究

大塚氏らは前述のRNA合成法を、新しい機能を持つRNAとして発見されたRNA酵素（リボザイム）の機能の研究に応用し、一九八七年以降多数の変異体合成を行うことにより切断反応に必須な塩基配列を見出し、塩基配列特異的RNA切断法を開発した（図4）。これを用いることにより、*ras*遺伝子からのメッセンジャーRNAのうち変異

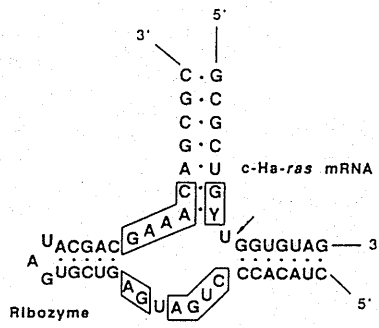



図4　RNA を配列特異的に切断するリボザイム

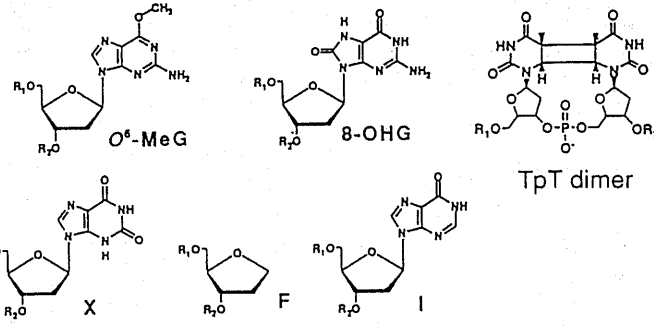



図5　損傷 DNA の構造

したもののみを切断し、その発現を阻害し得ることを示した。また、切断部位にホスホロチオエートを持つ RNA を合成することにより、リボザイムの切断反応がリボヌクレアーゼと同様の in-line 機構によることを証明した。

Ⅳ. *ras* 遺伝子と関連損傷遺伝子の機能

先に述べた合成 *ras* 遺伝子は微生物のコドンを用いて設計され、微生物中で正しい折りたたみの蛋白質が得られたが、この蛋白質の哺乳動物細胞における活性を調べるため一九八九年に NIH3T3 細胞への遺伝子導入を行った。哺乳動物細胞用のプロモーターを持つプラスミドを用いることにより、十二番目にグリシンを持つ正常 *ras* 遺伝子は細胞をがん化さ

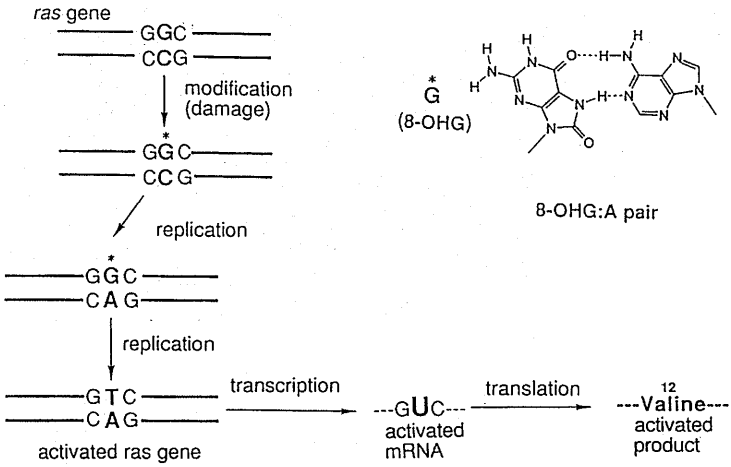



図6　GからTへの変異の機構



せず、十二番目がバリンまたは六一番目がロイシンに変異した活性型遺伝子は細胞をがん化させ、フォーカスを形成させることを明らかにした。このことにより *ras* 遺伝子の活性はその産物である蛋白質にあることを強く示唆することとなった。

次に、大塚氏らは *ras* 遺伝子の損傷が変異の原因となることを証明する実験を行った。図5に示す損傷塩基デオキシグアノシンのメチル化体（$O^6$-MeG）、水酸ラジカルによって生ずる8-オキシデオキシG（8-OHG）、デアミノ化されたデオキシキサントシン（X）、塩基が除去されたデオキシリボースのアナログ（F）を十二番目のグリシンの遺伝子 GGC の二字目に置換した。また、デオキシイノシンを六一番目グルタミンの遺伝子 CAA の二字目に置換した。更に相補鎖の TT 配列には紫外線によって生ずるチミンダイマーを置換した。これらの損傷塩基を含む *ras* 遺伝子を NIH3T3 細胞に導入し、がん化した細胞からの DNA を分析することにより変異を検出した。8-オキシデオシグアノシンに例をとると、GTC および GAC に変異したものが主として観察され、これは図6に示すよう

な異常な水素結合形成によるものであることを示した。

以上のように池原、大塚両氏は有機化学的手法を駆使して、RNA および DNA の分野で多くの成果を挙げ、広く世界的にその活動を知られている。此の事は両氏の国際的な多くの学会やシンポジウムでの招待講演にも現われており、これらの成果は三〇〇報を超える国際誌での論文に発表されている。

両氏は我が国の各種の学会の役員として多くの労をとり、また幾つかの国際学術雑誌の編集委員としても活躍している。

これらの業績は我が国の核酸科学の発展の一端を示すものとして高く評価できる。

主要論文リスト録

I. RNA 合成法の開発と tRNA 全合成の研究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