

THE p53 GENE EXPRESSION AND ITS DEVELOPMENTAL REGULATION IN SCHISTOSOMES

MANAMI TANAKA⁺; TADASHI MATSU-URA* & HIROHISA HIRAI**

Institute of Basic Medical Sciences, University of Tsukuba, Tsukuba, Ibaraki 305, Japan *Department of Parasitology, University of Shinshu, School of Medicine, Matsumoto 390, Japan **Department of Parasitic Diseases, Kumamoto University School of Medicine, Honjo 2-2-1, Kumamoto 860, Japan

We have studied the gene expression, especially of the oncoproteins, and its regulation in schistosomes. Schistosomes have a complex life cycle with a defined dimorphic lifestyle. The parasite are so far unique in biology in expressing oncogene products in their adult stage. In order to characterize the expression and developmental regulation, a lambda gt 11 cDNA library and lambda EMBL4 genomic DNA library of each growth stage of Schistosoma mansoni and S. japonicum was constructed, and was screened with various monoclonal antibodies against oncogene products. One positive plaque reacted to anti-p53 antibody (Ab-2, Oncogene Science, Inc.) was further analyzed. This fusion protein was about 120 KDa in molecular weights, and expressed as 1.4 Kb RNA in the adult stage. P53 gene is well-known as the negative regulator of the cell cycle, and the mutations in the gene are turning out to be the most common genetic alterations in human cancers. The comparison of the gene structure among species and stages were being conducted. Chromosome structures, C-band formation, and the results of in situ hybridization using the phage probe would be discussed.

Key words: schistosomes – oncoproteins – P53 gene expression

Recently, there has been a couple of reports concerning the unexpected discovery that schistosomes express the viral proteins (Tanaka et al., 1989; Khalife et al., 1990). Prof. Cox, King's College London, raised a good question to these discoveries in his review comments in *Nature* (1990), about the mechanism of acquisition and the function of those materials in schistosome infection. Generally speaking, it would be of great interest to know more about the molecules common to the schistosomes and their hosts. The recent advances in molecular biological techniques enabled us to get an abundant information through the genetic researches of the parasites, however, we must remember that there are so many "red herrings" in the process of the work which may easily lead us to the overestimation or misunderstanding of the data. Simpson & Pena (1991) also emphasized the necessity of skepticism in researcher mind to the discoveries which has not yet been well characterized.

Here we will describe the interesting and exiting process of our molecular biological approach to schistosomes. In 1987, we raised the first question, which was, "how schistosomes can disguise themselves in the long-term infection in mammalian hosts?" It seemed unlikely that schistosomes could share so many epitopes with host-related antigens as to be enough for the survival in years. The idea of the genomic incorporation of the mammalian genes were attractive, but also seemed unlikely since the simple comparison of the genome size clearly denied the possibility of the incorporation of a large molecules. The viral products, such as gp70, are rather small in size, and seemed plausible as a candidate of missing link between the host and the parasite.

Three laboratories examined the crossreactivity between the retrovirus products and schistosome antigens, first by immunohistochemistry (Tanaka and Uchiyama's group), second by Western blotting (Amanuma's group) using the same polyclonal goat antibodies, and the last by Southern blot analysis (Iwamura's group). These results were published in Para-

Sponsorship: Grant-in-Aid for Scientific Research (Project No. 63770242, No. 6380149, and 03807023) of the Ministry of Education, Science and Culture, Japan. The Grants from Uehara Memorial Foundation, Ohyama Health foundation, Nissan Science Foundation, and Ichiro Kanahara Foundation, Japan.

⁺Corresponding author.

sitology (Tanaka et al., 1989), however, the story has just begun. At the same time, one of the Pos. Doc. fellow in my laboratory obtained the puzzling data which indicates drastic rearrangements of DNA sequences in *Schistosoma mansoni* genomes in the course of development (Nara et al., 1990). It's impossible and also dangerous to conclude anything at this point for a simple explanation of these evidences, which urged us to make genomic DNA and cDNA libraries for further screening of the real virus genomes incorporated in schistosome genomes and their expression. Later, the DNA probes used in this examination was revealed to be the internal transcribed spacer region of rDNA gene population by *in situ* hybridization (Hirai et al., in press).

The genetic materials were obtained from adult female and male worms, and hatched miracidia of *S. japonicum* and *S. mansoni* by guanidinium/cesium chloride method (Sambrook et al., 1989). For constructing cDNA libraries, adaptor ligation (*EcoRI-NotI-BamHI* adaptor, Takara, Japan, or RibocloneTM *EcoRI* Adaptor Ligation System, Promega) method was employed instead of the cumbersome process of methylation and linker ligation after the cDNA synthesis (Amersham cDNA synthesis system plus). cDNAs were then size-fractionated by agarose gel, followed by arm ligation (*SfiI-NotI* Arms, Promega) and *in vitro* packaging (Gigapack^R II Gold Packaging extract, Stratagene). This method yields 10 times more efficiency per μg arm DNA, and 4 times less time. Furthermore, multiple restriction enzyme cutting sites incorporated in the arms will play an important role for further subcloning of the inserted DNA.

The phage plaques obtained were immunoscreened by various monoclonal antibodies (Oncogene Science, Inc.), by *picoBlue*TM Immuno-screening Kit (Stratagene). We picked up one positive plaque against anti-p53 monoclonal antibody (Ab-2) from *S. japonicum* adult male lambda gt11 cDNA library. The p53 gene is now well-known as tumour suppressor gene (Levine et al., 1991), which means the inactivation of this gene causes oncogenesis in tissue cells. The specific binding of wild type p53 to several viral oncoproteins, such as large T antigen of SV40, inhibits the native function of oncoproteins and suppress the oncogenesis in tissue and/or transfected cells. For quick detection of molecular weights of this fusion protein, top agar contains several plaques were

directly treated and used for SDS-PAGE analysis and Western blotting. However, by this method, high back grounds were also observed.

After confirming the specific bands against Ab-2 monoclonal antibody over high molecular weight markers, there were two ways to proceed the research. First, to obtain the high level expression of fusion vectors using plasmid vectors. Second, to sequence the insert DNA to define the molecular characters of the gene. The *SfiI-NotI* sites in the lambda gt11 phage arm was identical to pGEMEX-2 directional cloning vector (Promega). Using this system, the expression of the fusion protein of 120 KDa in molecular weights were clearly shown by Western blotting. Northern blot analysis using this plasmid DNA shows 1.4 Kb signals in adult worms, whereas no reactions could be observed in the RNAs extracted from hatched miracidia.

For sequencing, TA cloning kit (Invitrogen) was used. PCR amplified products from phage DNA were electrophoresed by agarose gel, and purified by centrifugation in C3HV filter (Millipore). These products were subcloned into specific plasmid vector in the kit, and after the transformation, a large amount of plasmid DNA was sequenced by autosequencer (Applied Biosystems) using dye termination method. Further analysis and comparison of the sequences and amino acids are now under investigation.

As genes are cloned in increasing numbers, the detection and characterization of the variants in genes has become a major area of interest in biology. Mutations, deletions and rearrangements, these variation may account for speciation, tumor formation, drug resistance, as well as the more obvious nature of infectious diseases. Sequence variation of the p53 gene has been reported to be responsible for the alteration in DNA replication, regulation of cell cycle, and interactions with other proteins such as hsp70. Originally, we were indicating to find the host-related factors in schistosome genomes, however, these results brought us into a big focus, the mechanism and factors involved in the regulation of complexed life cycle of schistosomes. Again, we must remember skepticism in our mind, and the biological and biophysical characterization of each factor, one by one, is the fastest way to understand evolutionary adaptation mechanism of parasites as well as general cell biology of parasitism.

REFERENCES

- COX, F. E. G., 1990. The worm and the virus. *Nature (news and views)*, 347: 618.
- HIRAI, H.; TANAKA, M. & LOVERDE, P. T., 1992. Karyotype map of chromosomes of *Schistosoma japonicum*. *Exp. Parasitol.*, in press.
- KHALIFE, J.; GRZYCH, J.-M.; PIERCE, R.; AMEISEN, J.-C.; SCHÄCHT, A.-M.; GRAS-MASSE, H.; TARTAR, A.; LECOCQ, J.-P. & CAPRON, A., 1990. Immunological Crossreactivity between the human immunodeficiency virus type 1 virion infectivity factor and a 170-kD surface antigen of *Schistosoma mansoni*. *J. Exp. Med.*, 172: 1001-1004.
- LEVINE, A. J.; MOMAND, J. & CATHY, A. F., 1991. The p53 tumour suppressor gene. *Nature*, 351: 453-456.
- NARA, T.; IWAMURA, Y.; TANAKA, M.; IRIE, Y. & YASURAOKA, K., 1990. Dynamic changes of DNA sequences in *Schistosoma mansoni* in the course of development. *Parasitology*, 100: 241-245.
- SAMBROOK, J.; FRITSCH, E. F. & MANIATIS, T., 1989. *Molecular cloning: A laboratory manual*, 2nd ed., Cold Spring Harbor Laboratory Press.
- SIMPSON, A. J. G. & PENA, S. D. J., 1991. Host-related DNA sequences in the schistosome genome? *Parasitology Today*, 7: 266.
- TANAKA, M.; AMANUMA, H.; IWAMURA, Y.; WATANABE, M.; WATANABE, T.; UCHIYAMA, Y. & YASURAOKA, K., 1989. Integration and expression of murine retrovirus-related sequences in schistosomes. *Parasitology*, 99: 31-38.