Most of it started with T4 phage and was then taken over

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Abstract Professor Fumio Arisaka is one of the famous leaders in bacteriophage research, especially in the areas of protein biophysics and structural biology. Autonomous phage morphogenesis is a self-assembly process controlled by subunit–subunit interaction. Under this principle, Fumio has studied T4 tail assembly and morphology. He has also contributed structural information about T4 phage through a combination of X-ray structural analysis and three-dimensional image reconstruction using cryo-electron microscopy. Most of the development of ultracentrifugation applications for molecular assembly and phage morphogenesis research was also performed in Fumio’s laboratory. Fumio is a pioneer of supramolecular protein assembly study, and his science continues in the research work of the approximately 150 people who had attended his final lecture at the Tokyo Institute of Technology.

Keywords Bacteriophage T4 · Self-assembly · Protein-protein interaction

The golden age of bacteriophage research

Many of the early studies in molecular genetics were started with “T-even” phages (i.e., T2, T4, and T6). For example, T-even phages were employed in studies decoding the genetic code (Crick et al. 1961) and to confirm that DNA is the genetic substance (Hershey and Chase 1952). Initially, electron mi-

croscopy was used to obtain the primary phage images (Brenner and Horne 1959). These pictures initiated extensive studies in morphological and structural biology, and led to the subsequent development and prosperity of cryo-electron microscopy. Indeed, the dawn of molecular biology was the golden age of bacteriophage research.

It is important for scientists to acquire suitable experimental materials for advancement of their research. Fumio Arisaka, a biophysicist with a strong interest in the mechanism of molecular assembly, was blessed with the bacteriophage T4. Fumio started his T4 phage research in Basel in 1977, and published on the purification of the T4 sheath subunit, gp18, in 1979 (Arisaka et al. 1979; Tschopp et al. 1979). Ever since this period, T4 phage has been the main research target for Fumio’s lifetime. T4 phage is composed of a head carrying the genomic DNA, a long and contractile tail with the baseplate, and long tail fibers. The head, tail, and long tail fibers are assembled independently before they are joined together to produce a mature T4 phage (Eiserling and Black 1994). Kikuchi and King showed that the molecular assembly of T4 phage occurred according to a well-ordered sequential process (Kikuchi and King 1975; King 1971). Moreover, it was very attractive for Fumio that reconstruction of the mature T4 phage for his assembly studies was made possible by using amber-defective mutant phages and quantitative infection experiments. The T4 sheath subunit can take three forms: monomer, extended sheath, and contracted sheath (Arisaka et al. 1981). The polysheath, which is a homopolymer of monomeric gp18, was known to be almost the same as the contracted sheath. Fumio was interested in not only the polymerization of the sheath subunits but also the difference between the two different polymerization states (i.e., the extended and contracted sheaths). Interestingly, it was believed that the T4 sheath was composed of 144 copies of gp18 (Coombs and Arisaka 1994) until Petr Leiman showed it to be 138 copies, using cryo-electron microscopy (Leiman et al. 2004).

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In 1980, Professor Shin-ichi Ishii of Hokkaido University (Sapporo, Japan) invited Fumio to join his laboratory staff, since Shin-ichi was interested in *Pseudomonas aeruginosa* pyocin R1 and its contraction. The structure of pyocin R1 is very similar to that of the phage contractile tail. One can say that the structural similarity between the T4 tail and pyocin R1 led to the encounter between Fumio and Shin-ichi and their subsequent cooperation. Thus, Fumio was back in Japan and started his T4 research in Sapporo. During that period, DNA sequencing was becoming popular. Fumio decided to determine the sequence of the sheath subunit gene (gene18) by using the Maxam–Gilbert method (Arisaka et al. 1988). For Fumio, the amino acid sequence information of gp18 was the key to investigating the fine molecular structure of the sheath and to understand sheath polymerization. Fumio also successfully purified the tail lysozyme, gp5 (Nakagawa et al. 1985).

My first encounter with Fumio Arisaka and T4 phage

It is very important and sometimes critical, for students to meet suitable supervisors. My first encounter with Fumio occurred in 1986, just after the determination of the gene18 sequence, when I was an undergraduate student at Hokkaido University. Fortunately, I was assigned as Fumio's student and tried to determine the sequences of gene5 and gene12. Sapporo is located in the north of Japan. It is very cold, and snow is abundant in winter. Nevertheless, the coldness of winter and the dry air were suitable for phage experiments, preventing contaminations by a young student. Fumio and I often conducted experiments together until nightfall, during which times he would talk about the overseas lifestyles of when he was a postdoctoral fellow. Fumio is an excellent flute player. He sometimes played for me. Fumio and I often discussed about the possibility of achieving the crystallization of gp18. The problem was how to prevent polymerization and formation of the polysheath under the crystallization conditions. Fumio's idea was to develop a limited proteolysis domain that had lost its polymerization activity (Arisaka et al. 1990). In my graduate course, I performed chemical modifications of the sheath to determine the surface residues of gp18 and the region hidden by polymerization. Chemical modification results showed that the N-terminal and C-terminal regions of gp18 were close to each other and were in an area important for polymerization (Takeda et al. 1990). These results were later demonstrated by X-ray crystallization analysis (Aksyuk et al. 2009). When Fumio joined the Evergreen Bacteriophage Conference during my graduate school days, I had a chance to accompany him. At the meeting, I had the opportunity to join in discussions with Jonathan King and other research pioneers. This experience decided my later research life. In 1988, I moved to the University of Tokyo to acquire expertise

in genetic engineering techniques. Fumio also moved at around that period, to take up an associate professor position at the Tokyo Institute of Technology. However, his research at the Institute was sometimes tough. The golden age of phage research had passed by then and molecular biology had shifted to mammal research. In 1996, I completed my postdoctoral fellowship and became a member of Fumio's laboratory staff. We employed classical mutation studies for T4 phage. Several interesting T4 mutants were available in Fumio's laboratory. Amber mutants were used for amino acid insertion experiments by using permissive hosts carrying several types of suppressor tRNA. Temperature-sensitive mutants (*ts*, which grow at 30 °C, but not at 42 °C) and heat-sensitive mutants (*hs*, which lose viability upon incubation at 55 °C for 30 min) showed stability. Cold-sensitive mutants (*cs*, which grow at 37 °C, but not at 25 °C) were believed to be related to subunit interactions. Carbowax mutants (*CBW*, which can adsorb to the host bacterium in the presence of a high concentration of polyethylene glycol, whereas wild-type phage cannot) reflected the infection process. These several phenotypes were advantages of phages as experimental materials. The relationship between the phenotypes of the mutant phage and the amino acid replacements was investigated in gene5 (Takeda et al. 1998b) and gene18 (Takeda et al. 2004). Fumio was well aware of these various mutants and their benefits.

The renaissance of bacteriophage research

At this time, Shuji Kanamaru joined Fumio's laboratory as a graduate student. I had to return to the University of Tokyo in 1998, whereas Shuji carried out his postdoctorate in Michael Rossmann's laboratory and succeeded in the crystallization of a gp5–gp27 complex (Kanamaru et al. 2002). This triggered a dramatic progress in the structural biology of phages. Structural analysis of supramolecular structures of several hundred nanometers was implemented by a combination of X-ray structural analysis and three-dimensional image reconstruction using cryo-electron microscopy (Belnap et al. 1999). Petr Leiman succeeded in reconstructing the three-dimensional image of T4 at Rossmann's laboratory (Kostyuchenko et al. 2003; Leiman et al. 2004). Then, it became possible to understand not only the overall shape of the phage but also the assembly and conformational changes in its infection process (Kostyuchenko et al. 2005; Leiman et al. 2010; Taylor et al. 2016). In addition to molecular biology, nanoscale structural biology was started with T4 phage, since the phage was optimal for this new nanobiology owing to its size, characteristic shape, and complexity. In this area, Fumio's knowledge and science were extremely important and decisive in the analysis and understanding of the obtained structure (Arisaka et al. 2016). When I got my own opportunity to head a laboratory in Gunma University in 2001, my target shifted from T4 phage to Mu phage. I applied almost the same strategies

used in Fumio's laboratory for the Mu phage experiments (Takeda et al. 1998a; Kitazawa et al. 2005; Kondou et al. 2005; Suzuki et al. 2010; Harada et al. 2013).

Fumio held and hosted the 1st annual bacteriophage meeting in Japan in 2006. After that, it was held every two years following the Evergreen T4 meeting. I organized the 4th annual bacteriophage meeting in 2012, to which I invited Petr Leiman. All four of us (Fumio, Shuji, Petr, and I) spent exciting days together (Fig. 1). Petr gave a presentation on the remarkable features of a phage DNA injection system, which works on the concept that it shares structural similarity with bacterial secretion systems that are used by pathogens to deliver their toxins into target eukaryotic host cells. Fumio gave a keynote lecture entitled “Upcoming and Future of Bacteriophage Meeting” in the 5th phage meeting in 2014. In these phage meetings, Fumio sometimes said, “The renaissance of phage research has come from structural biology.” This comment also meant that there was a dark era before the renaissance. Even in the dark ages before welcoming the renaissance, Fumio did not lose his science and research direction. Fumio also spread knowledge about ultracentrifugation analysis with the development of Beckman Coulter XL-I, and organized ultracentrifugation meetings several times in Japan. Determinations of molecular weight by ultracentrifugation give us decisive data for molecular assembly and phage morphogenesis research. For example, reconstitution of the hubless baseplate (Yap et al. 2010) and the neck ring (Akhter et al. 2007) using recombinant subunits was made possible by ultracentrifugation. The common feature of the bacteriophage and ultracentrifugation is that both are classical methods, but they had a forgotten decade owing to underestimation of their usefulness. Despite this tendency, Fumio had kept his eye on the goal and the most important thing for him in any time and in any age.

Fig. 1 Bacteriophage research engines in 2012, Gunma, Japan. From left: Petr Leiman, Shuji Kanamaru, Shigeki Takeda (author of this review), and Fumio Arisaka. The central signboard reads “The 4th Phage Meeting” in Japanese

We are successors

Most of us have learned many important things from T4 phage. The study expanded from molecular biology and structural biology to nanotechnology. Indeed, Fumio also contributed to nanotechnology research, although his interest was in basic biophysics. Most of the research fields (gene replication, gene organization, translation, self-assembly, host recognition, DNA injection, nanomachine construction, structural transformation of supramolecular organization, etc.) had started with T4 phage. The principle “autonomous morphogenesis is a self-assembly process controlled by subunit–subunit interaction” was realized by Fumio as “spontaneous collaboration is a self-organization process controlled by people–people communication.” Approximately 150 researchers gathered at Fumio's final lecture at the Tokyo Institute of Technology. Not only knowledge and techniques but also Fumio's scientific mind was passed on to these individuals. The research subjects of the lecture attendees were very diverse. It means that Fumio has contributed to widespread fields related with assembly and interaction studies. Fumio is a pioneer of supramolecular protein assembly study and his science lives on in the work of his myriad successors.

Compliance with ethical standards

Conflict of interest Shigeki Takeda declares that he has no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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