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Professor Hidesaburo Hanafusa: A 50-Year Quest for the Molecular Basis of Cancer

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A pioneer of oncogene research, Hidesaburo Hanafusa, Emeritus Director of the Osaka Bioscience Institute and Emeritus Professor of the Rockefeller University in New York, passed away on 15 March of this year in Japan. For his seminal works in cancer research during six decades (1950s until now), he received a number of awards including the Lasker Award, the Asahi Prize, the Ricketts Award, the Clowes Award, The Order of Culture (Bunka Kunsho in Japan) and the General Motors Sloan Award. I would like to provide a summary of his many contributions to research on viral and cellular oncogenes in order to appreciate the significance of his work on our understanding of the molecular basis of cancer and the implications for diagnosis and treatment.

Key words: recovered virus, Rous sarcoma virus, SH2/SH3, v-*crk*, v-/c-*src*.

Abbreviations: FSV, Fujinami sarcoma virus; OBI, Osaka Bioscience Institute; RSV, Rous sarcoma virus.

BEFORE THE MOLECULAR BIOLOGY OF RETROVIRUSES—THE DEFECTIVENESS OF ROUS SARCOMA VIRUS

Dr Hanafusa was born in Hyogo Prefecture Japan in 1929 and graduated from the Faculty of Science, Osaka University. He obtained a Ph.D. in Professor Shiro Akabori's laboratory and held a research associate position in the Institute of Infectious Disease at Osaka University. In 1961, Hanafusa went to the USA and joined the laboratory of Harry Rubin at the University of California, Berkeley, first as a postdoctoral fellow and then as an assistant research virologist. It was in Rubin's laboratory where he initiated his lifework—understanding of the molecular basis of cancer induced by Rous sarcoma virus (RSV), which was isolated in 1911 by Peyton Rous at the Rockefeller Institute.

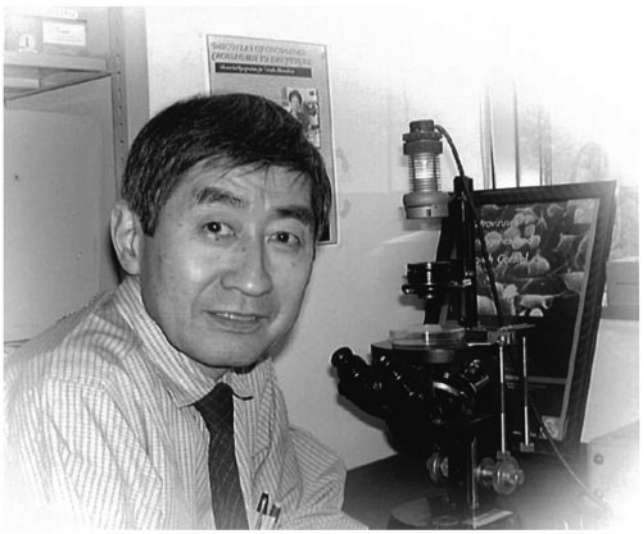
In Rubin's laboratory, Hanafusa published his first high-impact paper on the defectiveness of RSV along with his wife, Teruko Hanafusa, and Rubin as coauthors (*1*, *2*). Taking advantage of the 'focus assay' that had recently been developed in Rubin's laboratory that allowed for the quantitative analysis of cell transformation *in vitro*, Hanafusa clearly demonstrated that the ability of RSV (Brian high titer strain) to transform cells could be separated from its ability to replicate. This result provided one of the earliest pieces of evidence that the RSV genome contains a gene or genes for cell transformation that is not directly connected to viral replication.

TEMPERATURE-SENSITIVE MUTANTS OF RSV

In the late 1960s and early 1970s, three temperature-sensitive mutants of RSV were isolated. These studies further supported the hypothesis that there were genes capable of transforming cells in culture and inducing tumors in animals. Several laboratories including Hanafusa's newly established independent laboratory at the Public Health Research Institute, New York, generated temperature-sensitive mutants for the ability to transform. Kumao Toyoshima and Peter Vogt and then Steve Martin isolated a temperature-sensitive mutant of RSV (*3*, *4*). Hanafusa's group with collaborator Sadaaki Kawai isolated a widely used temperature-sensitive mutant of RSV named tsNY68 strain (*5*). These studies strongly implied that a product of the transforming gene was a protein with enzymatic activity.

RECOVERED VIRUS—TRANSFORMATION-POSITIVE MUTANTS RECOVERED FROM TRANSFORMATION-DEFECTIVE RSV MUTANTS

In 1976, the laboratories of Michael Bishop and Harold Varmus reported that v-*src* sequences are derived from



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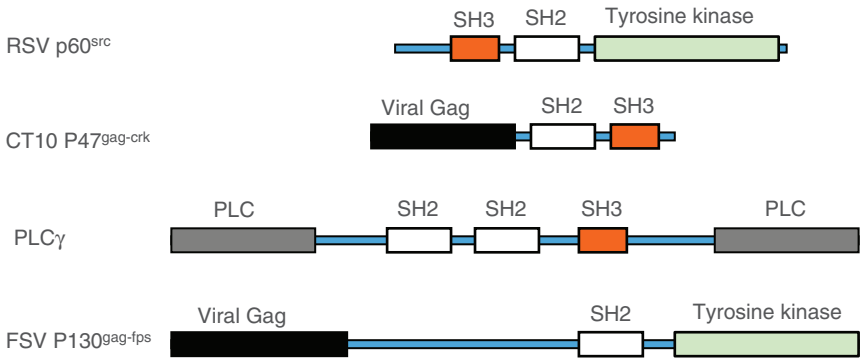

Fig. 1. **Structural relationship among v-*src*, v-*crk*, v-*fps*, and phospholipase Cγ.**

cellular gene sequences that were subsequently referred to as c-*src* (*6*). This critical observation was complimented by viral studies from the Hanafusa's laboratory. Hanafusa's group used chickens with transformation-defective RSV mutants and observed the formation of tumors with a long latency at sites distal to the site of inoculation. A transformation-positive virus was isolated from these tumors indicating that a transforming gene was 'recovered' by a recombination between viral and cellular sequences (*7–10*). The newly formed 'viral *src*' gene product had acquired several mutations that activated the enzymatic activity of the recovered *src* gene product (*11–16*). For this elegant and definitive study demonstrating how retroviruses obtain their oncogenes from normal cellular DNA through genetic recombination, Dr Hanafusa received the Lasker Award in 1982. The award citation stated: 'this finding was a major contribution and complemented the independent studies made by Drs. Bishop and Varmus'.

FUJINAMI SARCOMA VIRUS (FSV) CARRIES ANOTHER TYROSINE KINASE ONCOGENE

The *Src* gene product was characterized by Joan Brugge and Ray Erickson as a protein kinase (*17*), and Tony Hunter's laboratory discovered that the enzymatic activity specifically phosphorylated tyrosine residues on target proteins making v-Src the first protein tyrosine kinase (*18*). A question existed as to whether tyrosine kinases other than Src have cell-transforming activity. Hanafusa's group clearly showed that a viral Gag-fused oncoprotein of FSV isolated in Kyoto by Akira Fujinami in 1913 has a tyrosine kinase activity and is encoded by new tyrosine kinase gene named v-*fps*. His group also showed that v-*fps* in chickens is the homolog of the v-*fes* oncogene isolated from a mammalian virus. Thus, a conserved gene in chickens and mammals (c-*fps*/*fes*) had a strong oncogenic potential in both avian and mammalian species (*19–21*). With the publication of the sequence of the *fps* gene, it was observed by Tony Pawson that there were homologous regions that existed between v-*Src* and v-*Fps*, which he referred to as Src homology domains SH1 and SH2 (*22*). The significance of these domains would become apparent with the characterization of the transforming gene expressed by another retrovirus known as CT10.

THE v-*crk* IN CT10 SARCOMA VIRUS ENCODES A NOVEL TYPE ONCOPROTEIN, AN ADAPTOR PROTEIN

In the early 1900s, Peyton Rous isolated a series of chicken sarcoma viruses including CT1, which was a RSV. Later in the 1980s, Hanafusa's group extensively studied the oncogene of CT10, named v-*crk* and revealed that the Crk oncoprotein was an adaptor protein that consisted only of SH2 and SH3 domains (*23*). The SH3 domain, designated by Hanafusa's group, was another domain in Crk with homology with v-*Src* and v-*Crk* (Fig. 1). An organization of SH2 and SH3 domains was also found in another cell-signaling protein, phospholipase Cγ (*23*). Bruce Mayer and others in Hanafusa's laboratory revealed that the v-crk oncoprotein associates with phosphotyrosine-containing proteins and protein kinase activity (*24*, *25*). This observation, along with studies from Tony Pawson's laboratory provided a molecular explanation for the protein–protein interactions between SH2 domains and tyrosine-phosphorylated proteins, indicating a mechanism for a signaling cascade in cell transformation that involves tyrosine phosphorylation.

In 1998, Hanafusa left the Rockefeller University and returned to Japan as the Director of The Osaka Bioscience Institute (OBI). Since then, Hanafusa had continued an active research laboratory in the oncogene field not only on tyrosine kinases, but also on Crk, the serine/threonine kinase Akt, tumor suppressor genes and others (*26–35*). Three times since 2000, Hisataka Sabe and other members in OBI organized an international meeting on cell signaling. Hanafusa enjoyed vigorous discussions on the novel results presented by scientists from all over the world who were part of his laboratory at the Rockefeller University.

His many former students and post-docs feel most fortunate to have been a part of the science that emanated from the Hanafusa's laboratory and will continue his legacy worldwide.

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CONFLICT OF INTEREST

None declared.

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