

***Japan Academy Prize to:***

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and  
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for “Studies on Regulation of Eukaryotic Gene Expression using Bioactive Microbial Metabolites and their Application to Drug Discovery”

***Outline of the work:***

Approaching biological problems from the small molecule perspective presents a unique way of overcoming the limitations of classical genetics. The tremendous diversity of microbial metabolites provides a foundation to chemical biology. The discovery of new target-specific compounds contributes to the exploration of novel research fields and the unearthing of potential drug targets. However, a plethora of unique bioactive microbial metabolite targets awaits discovery. Prof. Minoru Yoshida has identified numerous microbial metabolites inhibiting eukaryotic gene expression processes through different and specific modes of action. These molecules have proven valuable biological probes, greatly enhancing our understanding of regulatory mechanisms in eukaryotic cells, and led to the development of novel medical therapies.

**1. Discovery of histone deacetylase inhibitors and their mode of action**

Murine erythroleukemia (MEL) cells resulting from an infection by the Friend virus usually differentiate into normal erythrocytes through treatment with dimethylsulfoxide (DMSO). Prof. Yoshida found that trichostatin A from a strain of the soil microorganism *Streptomyces* enhanced MEL cell differentiation more effectively than DMSO. A study on its mode of action revealed that this bioactive compound strongly inhibited histone deacetylase (HDAC).

Subsequently, Prof. Yoshida identified several structurally different microbial HDAC inhibitors including trapoxin, which facilitated the cloning of the first HDAC gene by covalent binding to cellular HDAC. These inhibitors have played a critical role in molecular studies on histone acetylation and its part in gene expression, contributing to the birth of a new research field called epigenetics. Moreover, Prof. Yoshida discovered that FK228, a microbial cyclic depsipeptide, was a natural prodrug inhibiting cellular HDAC through activation by the reducing intracellular environment. FK228 was approved for the treatment of T-cell lymphoma in 2009, emphasizing that research findings may lead to working medical treatments.

## 2. Discovery of protein nuclear export inhibitors and development of chemical genomics

Prof. Yoshida showed that chromosomal maintenance 1 (CRM1) acted as a target for the antifungal antibiotic leptomycin B (LMB), which induces morphological abnormalities in fungi. A highly conserved protein, CRM1 was originally identified as an essential protein regulating chromosome structure in fission yeast, but its molecular function was unknown. Prof. Yoshida and others demonstrated that CRM1 acted as a carrier for proteins bearing a nuclear export signal (NES) and that its covalent binding to CRM1 specifically inhibited protein nuclear export. Thus, LMB serves as an essential tool for analyzing protein trafficking from nuclei. Furthermore, because CRM1 is considered as a promising molecular target for anticancer drugs, several related inhibitors are currently in clinical development.

A collection of fission yeast strains with all individually tagged genomic open-reading frames (ORFs) was employed for a global analysis of CRM1-mediated protein nuclear export by LMB treatment. The global ORF expression platform also provided an innovative system to elucidate the mode of action of bioactive compounds. This system combining chemical biology and genomics methodology enabled the determination of the unusual mechanism of antifungal compounds known as theonellamides. Theonellamides were shown to bind to ergosterol in the fungal cell membrane, inducing abnormal  $\beta$ -1,3-glucan accumulation. This study demonstrated the effectiveness of chemical genomics.

## 3. Discovery of splicing inhibitors

In eukaryotes, protein-coding sequences, or exons, of nascent messenger RNA (pre-mRNA) are interrupted by intervening sequences called introns, which are eliminated from pre-mRNA by splicing. Prof. Yoshida revealed that the anticancer bacterial metabolite FR901464 was the first highly specific splicing inhibitor. By isolating proteins bound to spliceostatin A (SSA), a chemically stable derivative of FR901464, he identified the SF3b subcomplex of the spliceosome as the target. Surprisingly, some accumulated pre-mRNA species were translated into intron-containing proteins, indicating that the spliceosome normally helps retain pre-mRNAs within the nucleus and prevented their translation in the cytoplasm.

In summary, Prof. Yoshida plays a leading role in new biology fields, such as epigenetics, by elucidating the modes of action of novel bioactive microbial metabolites interfering with eukaryote-specific gene expression mechanisms. Furthermore, his work has unearthed new drug targets, leading to several inhibitors that have found practical use as anticancer drugs. Prof. Yoshida's achievements are acknowledged and celebrated around the world.

## List of Main Publications

### Original Papers

1. **Yoshida, M.**, Nomura, S., and Beppu, T. (1987) Effects of trichostatins on differentiation of murine erythroleukemia cells. *Cancer Res.*, 47: 3688-3691.
2. **Yoshida, M.**, and Beppu, T. (1988) Reversible arrest of proliferation of rat 3Y1 fibroblasts in both the G1 and G2 phases by trichostatin A. *Exp. Cell Res.*, 17: 122-131.
3. **Yoshida, M.**, Kijima, M., Akita, M., and Beppu, T. (1990) Potent and specific inhibition of mammalian histone deacetylase both *in vivo* and *in vitro* by trichostatin A. *J. Biol. Chem.*, 265: 17174-17179.
4. **Yoshida, M.**, Nishikawa, M., Nishi, K., Abe, K., Horinouchi, S., and Beppu, T. (1990) Effects of leptomycin B on the cell cycle of fibroblasts and fission yeast cells. *Exp. Cell Res.*, 187: 150-156.
5. Kijima, M., **Yoshida, M.**, Sugita, K., Horinouchi, S., and Beppu, T. (1993) Trapoxin, an antitumor cyclic

- tetrapeptide, is an irreversible inhibitor of mammalian histone deacetylase. *J. Biol. Chem.*, 268: 22429-22435.
6. Nishi, K., **Yoshida, M.**, Fujiwara, D., Nishikawa, M., Horinouchi, S., and Beppu, T. (1994) Leptomycin B targets a regulatory cascade of Crm1, a fission yeast nuclear protein, involved in control of higher order chromosome structure and gene expression. *J. Biol. Chem.*, 269: 6320-6324.
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  12. Kim, Y. B., Lee, K.-H., Sugita, K., **Yoshida, M.**, and Horinouchi, S. (1999) Oxamflatin is a novel antitumor compound that inhibits mammalian histone deacetylase. *Oncogene*, 18: 2461-2470.
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  14. Furumai, R., Komatsu, Y., Nishino, N., Khochbin, S., **Yoshida, M.**, and Horinouchi, S. (2001) Potent histone deacetylase inhibitors built from trichostatin A and cyclic tetrapeptide antibiotics including trapoxin. *Proc. Natl. Acad. Sci. USA*, 98: 87-92.
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## Reviews

1. **Yoshida, M.**, Horinouchi, S., and Beppu, T. (1995) Trichostatin A and trapoxin: novel chemical probes for the role of histone acetylation in chromatin structure and function. *Bioessays*, 17: 423-430.
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4. Seto, E., and **Yoshida, M.** (2014) Erasers of histone acetylation: The histone deacetylase enzymes. *Cold Spring Harb. Perspect. Biol.*, 6: a018713.