横川 隆志 (Takashi YOKOGAWA)

専門：Genetic engineering, protein engineering, RNA engineering

略歴：

昭和62年	3月	東京大学工学部工業化学科　卒業	
昭和62年	4月	東京大学大学院工学系研究科合成化学専攻修士課程	入学
平成元年	3月	同　上	修了
平成元年	4月	東京工業大学大学院総合理工学研究科生命化学専攻博士課程	入学
平成4年	3月	同　上	修了
平成4年	4月	東京工業大学助手（生命理工学部） （この間，現職のまま平成6年7月から平成7年11月まで ドイツ連邦共和国バイロイト大学で特別研究員）	
平成7年	12月	岐阜大学講師（工学部）	
平成15年	4月	岐阜大学助教授（工学部）	
平成19年	4月	岐阜大学准教授（工学部）	
平成25年	4月	岐阜大学教授（工学部）	
平成28年	5月	岐阜大学大学院連合創薬医療情報研究科・教授を兼務	
平成28年	10月	岐阜大学研究推進・社会連携機構生命の鎖統合研究センターを兼務	

研究課題：（代表的な研究）

1. Study on the synthesis of super proteins carrying unnatural amino acids inserted site-specifically

For site-specific insertion of unnatural amino acids into proteins, mutant tRNAs and mutant aminoacyl-tRNA synthetases—artificially introduced into the protein synthesis system—should not interfere with endogenous tRNAs or aminoacyl-tRNA synthetases. It was previously confirmed that tyrosine-tRNA and tyrosyl-tRNA synthetase from yeasts and methane-producing archaea can be introduced into Escherichia coli without interfering with the endogenous protein synthesis system. We have successfully created genetically modified tyrosyl-tRNA synthetase, which, unlike its wild-type counterpart, can recognize 3-azidotyrosine from yeasts and methane-producing archaea. In order to introduce unnatural amino acids in a site-specific manner, we also developed an efficient method for the preparation of an artificial amber suppressor tyrosine-tRNA, which can recognize amber (UAG) codons that usually serve as stop codons. Furthermore, by combining both, we have already succeeded in the site-specific introduction of 3-azidotyrosine, an unnatural amino acid, into proteins. Because the azide group is not a constituent functional group of natural polymers, chemical modification of introduced azide groups will add novel value to proteins. We have already achieved the following: modification with biotin or fluorescence residues without affecting the activities of the original protein; analysis of structural changes; and analysis of protein-protein interaction. Our preparation method for proteins carrying unnatural amino acids is a patented technology and will contribute to a number of collaborative studies.

2. Study of the structure-function relationship of modified nucleosides present in tRNAs

tRNAs play an important role in the conversion of the base sequences of genes into amino acid sequences of the corresponding proteins. One of characteristics of tRNA is the high content of modified nucleosides. Modified nucleosides appear to enhance the accuracy of codon recognition and the stability of 3D structure, thereby contributing to the fine-tuning of tRNA function. Against this background, we have been analyzing structures of modified nucleosides, and consequent changes in tRNA function. First, we found that addition of tetraalkylammonium salt increases the

tRNA-oligo DNA hybridization efficiency and we utilized this to establish a unique tRNA purification method. This efficient tRNA purification method successfully enabled purification of specific tRNAs and analysis of modified nucleosides that are contained within. For example, we discovered a modified nucleoside 5-formylcytidine in bovine mitochondrial methionine-tRNA and showed its involvement in the recognition of a non-universal genetic code (AUA for methionine instead of isoleucine) in mitochondria. We also discovered a novel modified nucleoside, agmatidine, in archaeal isoleucine-tRNA and found that it is essential in the recognition of the AUA codon. We are currently studying the mechanism involved in biosynthesis of archaeosine, an archaeal tRNA-specific modified nucleoside.

3. Study on the archaeal protein synthesis system

Archaea, lacking a nucleus, are classified as prokaryotes, but the characteristics of constituent factors of the protein synthesis system are more similar to those of eukaryotic cells than those of bacteria. Furthermore, by taking into consideration the number of constituent genes, the archaeal system has the appearance of a simplified system of the complex protein synthesis system in eukaryotic cells. However, all archaea are currently culturable only under extreme conditions (hyperthermophilic, halophilic, or acidophilic), and this has been an obstacle to investigating the protein synthesis system. We have selected several archaea that might grow at the temperature similar to that used for E. coli culture. Among them, Methanosarcina acetivorans, a methane-producing archaeon, is now culturable at the liter-scale. We are currently trying to establish a cell-free protein synthesis system and genetic engineering of this organism, and will study other archaea to establish the archaeal protein expression system.

研究業績：（過去５年の論文）

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   Escherichia coli-based production of recombinant ovine angiotensinogen and its characterization as a renin substrate. *BMC Biotechnol.*, **16**, 33 (2016)
2. Kawamura, T., Hirata, A., Ohno, S., Nomura, Y., Nagano, T., Nameki, N., Yokogawa, T., Hori, H.
   Multisite-specific archaeosine tRNA-guanine transglycosylase (ArcTGT) from *Thermoplasma acidophilum*, a thermo-acidophilic archaeon. *Nucleic Acids Res.*, **44**, 1894-1908 (2016)
3. Nomura, Y., Ohno, S., Nishikawa, K., Yokogawa, T.
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4. Inada, N., Nakamoto, K., Yokogawa, T., Ueno, Y.
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6. Ikeda-Boku, A., Kondo, K., Ohno, S., Yoshida, E., Yokogawa, T., Hayashi, N., Nishikawa, K.
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Involvement of plasmin-mediated extracellular activation of progalanin in angiogenesis. *Biochem. Biophys. Res. Commun.*, **430**, 999-1004 (2013)
8. Ikeda-Boku, A., Ohno, S., Hibino, Y., Yokogawa, T., Hayashi, N., Nishikawa, K. Site-selective post-translational modification of proteins using an unnatural amino acid, 3-azidotyrosine. *J. Biochem.*, **153**, 317-326 (2013)
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10. Yoshimura, S.H., Khan, S., Ohno, S., Yokogawa, T., Nishikawa, K., Hosoya, T., Maruyama, H., Nakayama, Y., Takeyasu, K. Site-specific attachment of a protein to a carbon nanotube end without loss of protein function. *Bioconjug. Chem.*, **23**, 1488-1493 (2012)

外部資金：（過去５年）

平成 23～25 年度文部科学省 (基盤研究C) 課題番号23570208
「tRNA スプライシング機構の特徴点抽出」

平成 24 年度岐阜大学研究活性化経費グループ研究支援
「機能未知タンパク質の機能解明ルーティンの確立」

特許：

１） 特許名：非天然蛋白質の製造方法、固定化方法及びキット
出願人：国立大学法人岐阜大学
発明者：西川一八、鈴木正昭、横川隆志、細谷孝充、大野敏
番号等：特願2003—5747（2003年3月4日　出願）
特許第3896460号（2007年1月5日　登録）

２） 特許名：部位特異的にタンパク質にチロシンアナログを導入する方法
出願人：国立大学法人岐阜大学
発明者：西川一八、横川隆志、大野敏
番号等：特願2007−521381（2006年6月14日　出願）
PCT/JP2006/312370
特許第4654449号（2011年1月7日　登録）

３） 特許名：Ｎ末端アミノ酸が標識されたタンパク質の効率的な合成方法
出願人：バイオコゥマー株式会社
発明者：金森崇、西川一八、横川隆志、大野敏
番号等：特願2007—545346（2006年11月16日　出願）
PCT/JP2006/323378
特許第5049136号（2012年7月27日　登録）

４） 特許名：タンパク質のＮ末を酵素的に修飾する方法
出願人：国立大学法人岐阜大学
発明者：西川一八、横川隆志、大野敏
番号等：特願2007—546539（2006年11月22日　出願）
PCT/JP2006/323879
特許第5061351号（2012年8月17日　登録）