DEPARTMENT OF VIRAL ONCOLOGY
LABORATORY OF CELL REGULATION

I. Sugita group

Full attention of the recently set up Sugita's laboratory has been directed to a novel lineage of antigen-presenting molecules, CD1. Unlike conventional MHC molecules that present protein-derived peptide antigens, molecules of the human group 1 CD1 family (CD1a, CD1b, CD1c) mediate presentation of lipid antigens to specific T lymphocytes. By taking cell biological and immunological approaches, this group wishes to establish a molecular and cellular basis for CD1-dependent immunity and determine how CD1 has been evolved to function critically in host defense. An important extension of this study is a challenge for developing a new type of lipid-based vaccines against cancer and microbial infection.

I-1) Development of appropriate animal models for CD1 study: I. MATSUNAGA, T. SHIINA* and M. SUGITA (*Tokai University)

Mice and rats are useful animals for many immunological studies, including those of MHC functions, but important exceptions exist. These animals have deleted genes for group 1 CD1 family, and thus, lack the CD1 system that is comparable to that in humans. Given the importance of appropriate small animal models in *in vivo* analysis of CD1-mediated immune responses, we have developed two distinct, but complementary, animal models; namely, guinea pigs and CD1-transgenic mice. We have found that guinea pigs have evolved the CD1 system equivalent to that in humans, and are capable of mounting the CD1-restricted T-cell response upon mycobacterial infection. Further, we have established methods for isolating CD1-positive dendritic cells from the guinea pig bone marrow. Using these as antigen-presenting cells, we are successfully obtaining guinea pig CD1-restricted T cells lines recognizing specific lipid antigens.

As an alternative animal model, we have tried to establish human CD1 transgenic mice in which CD1-dependent immunity is reconstituted. This year, we isolated the human genomic gene for CD1c and established CD1c transgenic mice. Strikingly, CD1c molecules in these mice appear to be expressed selectively in dendritic cells and a fraction of activated macrophages, as in humans. We are now attempting to knockout MHC genes in these transgenic mice to separately evaluate CD1 and MHC functions.

I-2) CD1-Dependent Immunity against *Mycobacterium avium* complex (MAC) and HIV-1: I. MATSUNAGA, A. OCHI and M. SUGITA

After moving to the Institute for Virus Research, the Sugita's laboratory has set out to study

CD1-dependent immunity against *Mycobacterium avium* complex (MAC). Infection with MAC is clinically important because of its resistance to treatment with antibiotics, and often critical in prognosis of AIDS patients. We established methods for purifying the natural form of glycopeptidolipid (GPL), a unique lipid component expressed in the cell wall of MAC, and detected the IgG antibody response directed against the sugar moiety of the compound in guinea pigs infected with MAC. Using this model, cellular and molecular mechanisms for the CD1-dependent humoral immunity are being studied.

Virus infected cells and cancer cells produce a wide array of unusual glycolipids and lipopeptides that could be monitored by CD1-rectricted T cells. In this aspect, we have just set up studies, specifically addressing how CD1 functions against HIV-1 infection and lung cancer.

I-3) Identification of a mite-derived lipid as a novel allergen for contact dermatitis: T. SASAI*, N. MORI*, I. MATSUNAGA and M. SUGITA (*Division of Applied Life Science, Kyoto University)

It is now clear that CD1 critically functions in host defense, but undesirable immune responses to lipids may result in tissue damage and autoimmunity. We found that monoterpene, α-acaridial, derived from astigmatid mite *Tyrophagus putrescentiae* induced allergic contact dermatitis, characterized by dermal vasodilation, infiltration of T lymphocytes and proliferation of keratinocytes. Studies using human peripheral blood T cells suggested that the specific response to α-acaridial occurred independently of NKT cells and γδ T cells.

II. Murakami group

Murakami's group is interested in the regulation of higher order chromatin structure and its role in various chromosome functions. Currently this group mainly focuses on the dynamic organization of heterochromatin that is important constituent of chromosome using fission yeast as a model organism. In addition, we analyze the regulation of DNA replication from a point of view of chromatin structure.

II-1) RNA polymerase II is required for RNAi-dependent heterochromatin assembly: H KATO, D. B. GOTO*, R. A. MARTIENSSEN*, T. URANO, K. FURUKAWA**, Y. MURAKAMI** (*Cold Spring Harbor Lab., USA, **Nagoya Univ.)

Heterochromatin is a transcriptionally inert higher-order chromatin structure that contributes to global transcriptional repression and genome integrity. In Schizosaccharomyces pombe, the RNA interference (RNAi) machinery converts pericentromeric non-coding RNAs into small interfering RNAs (siRNAs) and is required for the assembly of pericentromeric heterochromatin. Here we

describe a mutation in the second largest subunit of RNA polymerase II (RNAPII). Both wild-type and mutant RNAPII localized to the pericentromere. However, the mutation resulted in the loss of heterochromatic histone modifications, in the dislocalization of RITS (RNA-induced transcriptional silencing) complex and RDRC (RNA-directed RNA polymeraxe complex), and in the accumulation of pericentromeric non-coding RNAs, accompanied by the loss of siRNAs. This phenotype resembles mutants in RNAi and suggests that RNAPII couples pericentromeric transcription with siRNA processing and heterochromatin assembly.

II-2) Functional Studies of Chromatin Assembly Factors in Fission Yeast: K. DOHKE, S.I.S. GREWAL*, K. TANAKA, S. KATAYAMA***, T. URANO****, Y. MURAKAMI** (*National Institutes of Health, USA., **Shimane Univ., ***Saga Univ., ****Nagoya Univ.)

Higher order structure of chromatin plays an essential role in various nuclear processes. Once established, the higher order structure need to be maintained through cell division. The molecular mechanism for the maintenance of the chromatin structure is not well understood yet. Chromatin assembly factor-1 (CAF-1) is three subunits (p150, p60, and p48) complex and loads histone H3-H4 complex onto newly synthesized DNA through interaction with a replication factor, PCNA in vitro.

We identified fission yeast genes (*pcf1, pcf2, pcf3*) showing significant homology to CAF-1 p150, p60 and p48 subunits. We found that Pcf1, Pcf2 and Pcf3 form a complex that interacts with PCNA like human CAF1. We also showed that *pcf1*, *2* and *3* are required for maintenance of heterochromatin rather than establishment. It is known that human CAF-1 p-150 interacts directly heterochromatin protein HP1. In pombe, we found that fission yeast CAF-1 interacts heterochromatin protein Swi6 in S phase specifically.

Disruption CAF-1, however, does not affect cell growth significantly, indicating that other chromatin assembly factor(s) exist. Asf1 (anti-silencing function 1) is thought to be a histone chaperon working cooperatively with CAF-1. We analyzed the function of fission yeast Asf1 at heterochromatin and relationship with CAF-1 using temperature sensitive mutant, since *asf1* is an essential gene in fission yeast. Our results suggested that CAF-1 and ASF1 function independently to maintain heterochromatin. These date suggested that fission yeast CAF-1 and ASF1 does participate in "the exact inheritance of heterochromatin structure".

II-3) Chromodomain protein Swi6 recruits the histone deacetylase Clr3 to regulate gene silencing by pericentromeric heterochromatin: H. NAKAGAWA, M. HATTA, K. DOHKE, M. YOKOYAMA and Y. MURAKAMI

Transcriptionally silent heterochromatin is characterized by deacetylated histones. Histone H3 is deacetylated at Lys 9 and Lys 14 (H3-K9 and H3-K14), and methylated on Lys 9 (H3-K9), which is bound by a chromo-domain protein HP1. It is still unclear how histone tail modifications are orchestrated, and what the role of HP1 is in the formation of silent chromatin. It was thought that in the fission yeast *Schizosaccharomyces pombe*, deacetylation of H3-K14 by the histone deacetylase (HDAC) Clr3 was required for efficient methylation of H3-K9 by the histone methyltransferase Clr4, at pericentromeric heterochromatin. However, we found that neither Clr3 nor H3-K14 deacetylation was absolutely required for methylation of H3-K9 or the subsequent binding of the fission yeast HP1 homologue, Swi6. Instead, Swi6 was required for recruitment of Clr3, to deacetylate H3-K14, and both Swi6 and Clr3 were required for deacetylation of histone H4-K12. Since gene silencing was partially disrupted in the absence of Clr3, we propose that one of the roles of HP1/Swi6 in transcriptional gene silencing, is enhancement of histone tail deacetylation by recruiting HDACs such as Clr3.

II-4) Analysis of a Novel Gene that May Involved in Repair of Double Stranded DNA Break during DNA Replication: M. YOKOYAMA, H. INOUE*, C. ISHII* and Y. MURAKAMI (*Saitama Univ.)

Neurospora crassa mus-7 mutant is sensitive to killing by MMS but not sensitive to UV radiation, suggesting that the gene product is involved in DNA double-strand breaks repair pathway. We cloned the *N. crassa* gene and sequenced the DNA. We found a gene in *Schizosaccharomyces pombe* showing significant similarity to *N. crassa mus-7* and named the gene *mus7*. We disrupted the gene. Like *N. crassa mus-7* mutants, the *S. pombe ⊿mus7* mutants are sensitive to MMS but not sensitive to UV radiation. Earlier study reported that there is a similar protein in *Saccharomyces cerevisiae* in respect of the amino acid sequence and the phenotype of the mutants. We have been analyzing the function of the gene using *S. pombe* Δ *mus*7 mutants. Our current results indicates that Mus7 may be involved in repair of double stranded DNA break during DNA replication, therefore we are finding out the relationship between *mus*7 and known repair genes working during DNA replication.

II-5) Regulation of the loading of *Drosophila* ORC1 (DmORC1) onto the chromosomal DNA replication origin for gene amplification: H. KOHZAKI and Y. MURAKAMI

A large body of evidence shows transcription factors affect DNA replication. We previously indicated that transcription factors regulate the origin activity by recruiting initiator proteins onto replication origins using polyomavirus and yeast system. We assume that this phenomenon is one of

many mechanisms leading to chromosomal abnormality including gene amplification, translocation, which are commonly observed in cancer cells. We wish to know whether DNA binding proto-oncogene products can regulate the chromosomal DNA replication in higyer eukaryote. But chromosomal origins of higher eukaryotic cells are not still characterized in detail. *Drosophila* (Dm) chorion gene amplification is a good model system, because the gene amplification is strictly regulated during development and utilizes similar set of proteins to normal chromosomal replication, such as origin recognition complex (ORC). The replication origin for the amplification contains two essential elements *ACE3* and *ori-beta* where DmOrc2 binds. Interestingly, transcription factors, such as E2F and Myb, regulate this amplification.

We first analyzed the behavior of DmORC1, because unlike DmORC2, the level of DmORC1 is modulated during cell cycle and its concentration regulates origin utilization during development. We indicated that DmOrc1-GFP formed foci in the follicle cell nucleus, which probably represents the amplifying loci. ChIP assay using Dm Egg chamber indicated that DmOrc1 bound to ACE3 and Ori-beta. Considering the regulatory role of DmORC1, we speculate that loading of DmORC1 is a major regulatory step for the gene amplification. We hypothesize that the transcription factors regulate the loading of DmORC1, because we found a transcription factor controls the loading of ORC to the replication origin in yeast. In addition, overexpression of DmORC1 induced abnormal DNA replication like overexpression of Cdc18/CDC6 in fission yeast. This may represent the functional exchangeability between ORC1 and CDC6. We are now trying to determine the function of these proteins at the chorion gene in *Drosophila*.

LIST OF PUBLICATIONS
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Laboratory of Cell Regulation

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がんウイルス研究部門　　Department of Viral Oncology
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I. 杉田グループ

2004 年 9 月に杉田が着任し、同年 12 月には助手の松永が着任した。CD1・脂質免疫応答の研究を担う新しい研究室のセットアップはまさしくゼロからのスタートであったが、所内外多方面からの協力を得て、ようやく機能的な研究室としての体裁を整えた一年であった。無菌培養室や生化学実験室、さらにモルモットや CD1 トランスジェニックマウスを用いた抗酸菌感染動物実験室の整備も完了し、徐々に成果を挙げつつある。ＬＣやＭＳシステムが手元にないのはまことに不便であるが、学外共同研究者の理解を得て、菌体やがん細胞からの脂質の精製および構造解析もようやく進められるようになってきた。大学院生を受け入れる態勢も整い、2006 年 4 月から、生命科学研究科と医学研究科より数名の大学院生が新たに参加する予定である。

そのようなセットアップ途上の研究室に 2005 年 7 月より加わった京大総合人間学部 4 回生の越智昭仁君は、松永助手のマンツーマンの指導を受け、短期間のうちに *Mycobacterium avium* complex (MAC)感染モルモットの解析基盤を確立した。そして、MAC 特異的な複合糖脂質である glycopeptidolipid (GPL)を、何ら人工修飾を受けていない天然型として単離精製することに成功し、それを用いて GPL 対する獲得免疫の存在を実証するとともに、その免疫認識エピトープを同定した。このモデルシステムは、今後の CD1 依存性生体防御機構の成立基盤の解析において極めて有用である。

また、CD1 が宿主防御に重要な役割を果たしている反面、脂質に対する過剰あるいは不適切な免疫応答は、アレルギーや自己免疫疾患の発症に繋がるであろうとの視点から、接触皮膚炎やギランバレー症候群の研究を展開し、成果を挙げた。とりわけ、農学研究科森助教授研究室より当研究室にやってきた笹井俊雄君（修士 2 年）は、ダニ由来脂質であるα-acaridial を精製し、この物質が接触皮膚炎のアレルゲンとして機能することを実証した。現在、このα-acaridial に対する免疫応答に関わる細胞の同定とその活性化制御法の確立を進めている。

当研究室が目指す、免疫学・細胞生物学・脂質生化学の融合とそれを基盤にした新しい研究領域の開拓ならびに医学への応用は、まだその緒に就いたばかりではあるが、オリジナリティーの高い研究成果が少しずつ得られつつある印象である。

II. 村上グループ

2005 年春に生命科学研究科修士課程を終えた上田は進路を変えて記者として新聞社に、同じく八田は医学研究科の博士課程へとそれぞれ巣立っていった。２人の新天地での活躍

を祈っている。新しいメンバーは加わることなく、残った総勢５名の小世帯でそれぞれの課題に取り組んできた。

そんな中この一年の最大の収穫は大学院生の加藤太陽の研究成果が Science 誌に掲載されたことであろう。加藤は博士後期課程入学後自ら発案したヘテロクロマチン形成に関わる新規変異株のスクリーニングに取り組んできた。よりよいスクリーニング方法の模索や変異株単離後の原因遺伝子のクロニーングなど、多くの壁に突き当たりながら、３年以上にわたり、ねばり強く解析を進めてきた。その結果、セントロメアヘテロクロマチンが特異的に崩壊する変異株のひとつが RNA ポリメラーゼ II のコアサブユニットに変異を持つことを見つけた。この変異は通常の転写にはほとんど影響を与えない。セントロメアヘテロクロマチンは RNAi システムに依存して形成されることが最近わかり、注目を集めているが、加藤の単離した RNA ポリメラーゼの変異株では RNAi システムがうまく機能してないことが判明した。この結果は RNAi システムさらにヘテロクロマチン形成に RNA ポリメラーゼが深く関わることを示した重要な知見であり注目を集めている。さらに、この知見は最近話題の non-coding RNA の機能を考える上でも重要な示唆を与えるだろう。この加藤の成果は改めて、正攻法の遺伝学的アプローチの強みと、研究を進める上でのねばり強さの重要性を教えてくれた。今後、この成果をさらに発展させていきたい。

他のメンバーの研究も着実に進行しつつある。道家はクロマチン構造の維持に重要なヒストンシャペロンの CAF1, ASF1 の解析を続けており、これらの因子がヘテロクロマチンの維持においてそれぞれ独立に機能していることを明らかにしてきた。クロマチン構造の維持機構はまだほとんど未解明であり、道家はその分子機構に迫ろうとしている。また、研究のかたわら、学生を中心としたウイルス研究所学術交流会の責任者として奔走し、見事交流会を成功に導いた。研究との両立に悩んだこともあったようだが、この経験は今後生かされるはずである。横山は学部生時代に自分自身が見つけたアカパンカビの DNA 修復関連遺伝子の分裂酵母ホモログの解析を続けてきた。今までの解析結果はこの遺伝子産物が S 期の DNA 損傷の修復に重要な機能を果たすことを示している。特に組み換え修復経路とチェックポイント経路に関連している可能性が強く、ゲノムの安定維持の観点から興味が持たれる。道家、横山ともに博士課程の最終学年を迎えるのでそれぞれ、成果を論文にまとめようと額に汗を流して頑張っている（はず）である。

ポストドクターの神崎は転写因子と染色体複製制御の関連について地道に研究を重ねてきており、その成果をレビューにまとめ発表した。現在は特にゲノムの安定性と複製の関連観点からショウジョウバエの遺伝子増幅システムに興味をもち、その制御機構、特に転写因子による制御に着目して解析を進めている。ショウジョウバエは我々のグループでは初めてのシステムであり、神崎は京都工芸繊維大学の山口研究室、京都大学生命科学研究科の上村研究室さらにノースカロライナ大学の浅野真樹博士の助力を受け、精力的に解析を進めており、興味深い結果が得られつつある。