Ilho Ha* and Gary Ruvkun
Department of Molecular Biology,
Massachusetts General Hospital, Harvard
Medical School, Boston 02114, U.S.A.
*Present address: ARS Network Center, SKKU,
Suwon 440-746, Korea

Caenorhabditis elegans is one of the ideal organisms to study the heterochronic genes. A heterochronic gene in *C. elegans*, *lin-14*, generates a temporal gradient of the LIN-14 proteins to control stage-specific patterns of cell lineage during development. Down-regulation of LIN-14 is mediated by the *lin-14* 3' untranslated region (UTR), which bears seven sites that are complementary to the regulatory *lin-4* RNA. We found molecular and genetic evidence that RNA duplexes between the *lin-4* and *lin-14* RNAs form *in vivo* and are necessary for LIN-14 temporal gradient generation. Four of the seven *lin-4/lin-14* RNA duplexes are predicted to bulge a *lin-4* C residue, and three sites are predicted to form nonbulged RNA duplexes. Reporter genes bearing multimerized bulged C *lin-4* binding sites show almost wild-type temporal gradient formation, whereas those bearing multimerized nonbulged *lin-4* binding sites do not form a temporal gradient. Interestingly, *lin-4* RNA binds *in vitro* to nonbulged *lin-14* RNA more avidly than to the bulged *lin-14* RNA. This suggests that a specific secondary structure of *lin-4/lin-14* RNA duplex that may be recognized by an accessory protein, rather than an RNA duplex *per se*, is required *in vivo* for the generation of the LIN-14 temporal gradient. We are currently searching for the factors interacting with the RNA duplex.

SL803

Mitochondrial DNA Mutation Analysis Using Paraffin-embedded Muscle Tissues

Sang Ho Kim
Department of Biology, Taegu University,
Kyungsan 712-714

A variety of mitochondrial DNA (mtDNA) defects, ranging from point mutations and large-scale deletions to severe reduction in the overall quantity of mtDNA (mtDNA depletion), may be associated with human mitochondrial diseases. More than 50 pathogenic point mutations and a myriad of rearrangements (deletions, duplications, or both together) have been described over the past 12 years, after the first pathogenic mtDNA mutation was reported to be associated with human disease in 1988. In this disorder, the population of wild-type and mutant-type mtDNA molecules coexists, a situation known as "heteroplasmy". The exquisite sensitivity of PCR has afforded molecular studies of fixed paraffin-embedded tissue specimens, which comprise most archival clinical material. Detailed genetic studies are now becoming feasible using these archival materials. We extracted DNA from these paraffin blocks from MELAS and KSS patients and PCR was carried out to analyze the mtDNA mutations (point mutation in MELAS and large-scale deletion in KSS). We did PCR-RFLP (HpaIII digestion) on MELAS which is an A -> G transition at position 3243 in tRNA-Leu (UUR) gene and could find the heteroplasmic nature of this MELAS mutation. In the case of KSS patients,

we used three primers ("3-primer PCR") to amplify in parallel wild-type and deleted mtDNA ("common deletion") and two characteristic fragments of wild-type and common deletion could be detected. Quantitation of these mutations is being done to see (possible) quantitative correlation between the amount of mutation and clinical severity. The present findings serve to emphasize the extent to which formalin-fixed paraffin sections represent a valuable repository of genetic material for a molecular genetic study.

SL804

Y Chromosomal DNA Variation in East Asian Populations and its Potential for Inferring the Peopling of Korea

Wook Kim*[1], Dong Jik Shin[1], Shinji Harihara[2] and Yung Jin Kim[3]

[1]Department of Biology, Dankook University, Cheonan 330-714, Korea; [2]Department of Biological Sciences, University of Tokyo, Tokyo 113- 0033, Japan; [3]Department of Biology, Chungnam National University, Taejon 302-764, Korea

We have examined variation of five polymorphic loci (*DYS287, DXYS5Y, SRY465, DYS19* and *DXYS156Y*) on the Y chromosome in samples from a total of 1,260 males in eight ethnic groups of East Asia. We found four unique haplotypes constructed from three biallelic markers in these samples of East Asians. The Japanese population was characterized by a relatively high frequency of either the haplotype I-2b (*-/Y2/T*) or II-1 (*+/Y1/C*). These dual patterns of the distribution of Y chromosomes (I-2b/II-1) were also found in Korea, although they were present at relatively low frequencies. The haplotype II-1 was present in Northeast Asian populations (Chinese, Japanese, Koreans and Mongolians), with the exception of a single male from the Thais in the Southeast Asian populations (Indonesians, Philippines, Thais and Vietnamese). The Japanese were revealed to have the highest frequency of this haplotype (27.5%), followed by Koreans (2.9%), Mongolians (2.6%) and mainland Chinese (2.2%). In contrast, the haplotype I-2b was found to be in the Japanese (17.1%), Indonesian (9.5%), Korean (6.3%), Vietnamese (3.8%) and Thai samples (2.7%). These findings suggested that the chromosomes of haplotype I-2b were likely derived from certain areas of Northeast Asia, the region closest to Southeast Asia. Phylogenetic analysis using the neighbor-joining tree also reflected a general distinction between Southeast and Northeast Asian populations. The phylogeny revealed a closer genetic relationship between Japanese and Koreans than to the other surveyed Asian populations. Based on the result of dual patterns of the haplotype distribution, it is more likely that the population structure of Koreans may not have evolved from a single ancient population derived from Northeast Asians, but through dual infusions of Y chromosomes entering Korea from two different waves of East Asians.