2015 Joint Usage and Research Report

Title of Research Project		Structural and functional analyses of nuclear RNA granules in mammalian cells
Applicant	Institution	Institute for Genetic Medecine
	Job title and name	Professor Tetsuro Hirose
Visiting researcher	Name	Dr Gerard PIERRON, Research Director. Centre National de la Recherche Scientifique (CNRS) - France.
Purpose of the Research Project (approx. 250 words)		**Structural and functional analyses of RNA-dependent nuclear bodies in human cells.** Paraspeckle nuclear bodies are formed around an architectural long non coding RNA, NEAT1, which is transcribed as two distinct isoforms from a unique promoter. Our groups, independently, previously showed that both isoforms have a distinct spatial distribution within the paraspeckles and a distinct role in their formation. The short isoform (3 700 nt) is only found at the periphery of the bodies and is not sufficient for their assembly when introduced into a NEAT1 KO cell. This contrasts with the ability of the long isoform (22 700 nt), the central region of which is found within the bodies, to generate paraspeckles in NEAT1 null cells. Paraspeckles are unique among nuclear structures as being cylindrical with a constrained diameter (rather than roundish). We hypothesized that their shape and limited diameter was dictated by NEAT1 as a long, looped and folded transcript. In a collaborative study, we further showed that, upon proteasome inhibition, transcription of both isoforms was enhanced, leading to paraspeckle expansion by elongation with an unvarying diameter. In this setting, we demonstrated that paraspeckles behave as “molecular sponges”, regulating gene expression by sequestrating regulatory factors. Our research program is two fold: (i) create partial NEAT1 deletion mutants by CRISPR/Cas9 technology and analyze at high resolution the effects that are resulting on the assembly, the ultrastructure, the size and the function of the paraspeckles (ii) define ultrastructurally nuclear bodies newly-identified in Sapporo, that contain essential RNAs and protein components (some being cancer-related factors) and that, depending on the cellular context, are seen as fused or separate entities. This second project is technically reminiscent of our recent

	demonstration of the formation by fusion of hybrid PML/p62-SQSTM1 bodies in Hela cells by electron microscopy (Souquere S. et al, Nucleus 6:326-38, 2015). Defining the interactions between these nuclear bodies will help to discriminate between different models of nuclear body formation like self-organization (random order of association of components), liquid droplet behavior or ordered assembly with defined molecular seeds.
Development of the Research Project and Results (approx.. 850 words)	We have carried out a series of collaborative experiments since 2012 leading to major progresses in paraspeckle knowledge. In short, combining molecular and microscopic analyses we have demonstrated the exquisite sensitivity of the NEAT1 lncRNA and of the paraspeckles towards proteasome inhibition. This provided us with a paradigm of paraspeckle expansion, enhancing greatly their function so that half of the nucleoplasmic content of major regulatory factors such as NONO and SFPQ was displaced and sequestrated in the paraspeckles. This resulted in major changes in gene expression as measured by us and by another group (with another inducer of paraspeckle expansion). We also identified the SWI/SNF chromatin-remodeling complex as a surprising paraspeckle assembly factor. This result was a direct consequence of the identification by the Hirose's group of 35 paraspeckle protein components, a number of which turn out to be classified as SWI/SNF interacting proteins in proteome databases. As a follow up, it was shown that silencing of both BRG1 and BRM SWI/SNF ATPases was leading to paraspeckle disintegration. Disentangling interactions between essential assembly factors like the lncRNA NEAT1 and essential protein components such as NONO, SFPQ, SWI/SNF is crucial to implement our understanding of the molecular interactions at play in paraspeckle assembly. During my visit at the Institute of Genetic Medicine of the Hokkaido University in Sapparo (August 19-September 10, 2015), at the invitation of Professor Tetsuro Hirose, we initiated a series of collaborative experiments, combining molecular and structural approaches, like analysis of NEAT1 deletion mutants by immuno-fluorescent microscopy, super resolution microscopy and immunogold electron microscopy. Various partial deletion

	mutants of the long NEAT1 isoform were created in Sapporo and mutated cell lines scrutinized under the EM to pinpoint resulting changes on paraspeckle morphology. HAP1 cells have been used because of their haploid chromosome content. We defined paraspeckle geometry in this cell type and we checked that they were sensitive to proteasome inhibition. In doing so, we have noticed the formation of membrane-less cytoplasmic bodies that contain a variety of paraspeckle proteins. Since some paraspeckle components such as FUS or TDP43 are prone, in certain circumstances, to generate pathogenic cytoplasmic aggregates, we are further analyzing the stress conditions leading to the formation of these cytoplasmic "bodies" and their relationship, if any, to ubiquitous membrane-less cytoplasmic structures like the P-bodies. Our results confirm that the long NEAT1 isoform is essential for paraspeckle assembly and reveal that the paraspeckle diameter is greatly reduced by the deletion of central regions of this lncRNA. In doing so, we have defined the genetic determinants that are shaping the paraspeckles, with a length depending on NEAT1 abundance and a diameter determined by NEAT1 size. In parallel, we have defined ultrastructurally, by immunogold electron microscopy, one of the 2 nuclear bodies that are seen as separate entities in the U2OS osteo-carcinoma derived cell line and as a single body in cervical cancer derived HeLa cells. In the absence of an efficient antibody to characterize the second body under the electron microscope, we used GFP-fusion proteins as markers. However, we are facing unpredicted technical difficulties in this project. It is still not yet clear whether chemically-fixed cell samples were "damaged" by transportation between Japan and France or whether there is an intrinsic difficulty as is occurring when GFP epitopes are masked by the fused protein and therefore not accessible to antibodies. We are actively pursuing this study that will help to characterize the dynamic relationship of these bodies in tumoral cells. Recent reports are also suggesting that NEAT1 and the paraspeckles are markers and potential prognostic factors for cancer development. Our studies will improve our knowledge of the functional and structural properties of cancer cells. Our most recent results are not yet published but will likely be in the next few months.

Publication *Enter the names of conference or journal and its vol. No. where the above work was presented.	【Conference, symposium, workshop etc.】
	【Journals】 Kawaguchi T, Tanigawa A, Naganuma T, Ohkawa Y, Souquere S, Pierron G, Hirose T. SWI/SNF chromatin-remodeling complexes function in noncoding RNA-dependent assembly of nuclear bodies. *Proc Natl Acad Sci U S A.* 2015; 112: 4304-9. T. Hirose, G. Virnicchi, A. Tanigawa, T. Naganuma, R.Li, H. Kimura, T. Yoko S. Nakagawa, M. Benard, A. Fox, and G. Pierron NEAT1 long noncoding RNA suppresses transcription via protein sequestration within subnuclear bodies. *Mol Biol Cell* 2014; 25:169-83.