

**P013** Subcellular distribution analyses of HD-PTP  
**Fumitaka Ichioka, Mayumi Okumura, Hideki Shibata,  
Vladimir L. Buchman and Masatoshi Maki**

*Department of Applied Molecular Biosciences, Graduate  
School of Bioagricultural Sciences, Nagoya University,  
Japan*

Mammalian Alix is a multifunctional adaptor protein involved in cell death, receptor endocytosis, endosomal protein sorting and cell adhesion by associating with various proteins such as ALG-2, CIN85/Ruk<sub>1</sub>/SETA, endophilins, CHMP4s and TSG101. In mammalian cells there are two other Alix paralogues, Brox and HD-PTP. HD-PTP is a putative protein tyrosine phosphatase (PTP) that contains a Bro1 domain, a V domain, a proline-rich region (PRR) in addition to a PTP domain. Previously we investigated interactions between HD-PTP and Alix-binding proteins. In the yeast two-hybrid assay and the Strep-pulldown assay, HD-PTP showed positive interactions with CHMP4b, TSG101, endophilin A1 and ALG-2 but not with either RabGAPLP or CIN85/Ruk<sub>1</sub>/SETA. In this study we analyzed the subcellular distribution of HD-PTP using monomeric green fluorescent protein (mGFP)-fused HD-PTP. In HeLa cells, mGFP-HD-PTP was distributed in the punctate manner and diffused in the cytoplasm, and mGFP-HD-PTP puncta was co-localized with early endosome markers (EEA1 and HRS) but not with late endosome/lysosome markers (LBPA and LAMP1). Microscopic analysis using the truncated mutants of mGFP-HD-PTP indicated that PRR plays an important role in the localization of mGFP-HD-PTP to endosomes. Further analysis using HD-PTP mutants would reveal the localization mechanism of HD-PTP that is needed for its function.