Introduction. Antibodies are Y-shaped glycoproteins made by humans and animals. They have the ability to recognize and discriminate among very similar foreign proteins. Nowadays, many monoclonal antibodies are produced in vitro by bulk tissue cultures of selected mouse hybridoma cell clones, but, despite their high specificity, for immunogold electron microscopy rabbit polyclonal antibodies are preferentially used. Preparation of cells as cryo sections for transmission electron microscopy (TEM) represents a method where cell proteins preserve their antigenicity well. This gives an opportunity that also monoclonal antibodies can be used for efficient immunogold labelling. In this study labelling signal and specificity of mouse monoclonal antibody against human endogenous inhibitor cystatin C have been compared to polyclonal antibody by fluorescence and electron microscopy. By applying monoclonal antibody on cryo sections of Escherichia coli cells expressing recombinant cystatin C (1) it was tested whether this antibody recognizes the protein of interest and whether it can be used for TEM.

Materials and methods. Escherichia coli cells were fixed with 4% paraformaldehyde, directly added to culture media at cultivation conditions. For fluorescence microscopy cells were cytocentrifuged at 265×g to glass slides and labelled with primary polyclonal and monoclonal anti-cystatin C and secondary Alexa Fluor-labelled antibodies. For TEM cells were embedded into 10% gelatin, infused with 2,3 M sucrose, preserved in liquid nitrogen and cut to ultrathin cryo sections (70 nm) at -120°C. Cryo sections were labelled with specific anti-cystatin C antibodies and Protein A-gold (10 nm). In case of primary mouse monoclonal antibody a linker rabbit anti-mouse antibody was added prior to Protein A-gold. Sections were contrasted with uranyl acetate and viewed in Philips CM120 Biotwin microscope. Distributions of 200 gold particles were compared in 3 compartments: periplasm, inclusion bodies and cytoplasm.

Results and discussion. By using immunofluorescence microscopy no significant differences in recombinant protein intracellular localization were observed after labelling with polyclonal and monoclonal antibody (Fig. 1). On the contrary, by using TEM (Fig. 2) it was clearly observed that polyclonal antibody recognized cystatin C in inclusion bodies (insoluble aggregates of recombinant cystatin, most probably inactive and misfolded) and in cell periplasm, whereas monoclonal antibody recognized predominantly recombinant protein in cell periplasm (soluble, properly folded recombinant protein, 72% of a total signal, Fig. 3). In conclusion, tested monoclonal antibody against human cystatin C works well on cryo section prepared for TEM.

This work was performed at EMBL Heidelberg and supported by EMBO fellowship (ASTF 90.00-05) to T.Z.B.

Reference
Fig. 1. Human recombinant cystatin C, expressed in *Escherichia coli*. Fixed cells were labelled with primary rabbit polyclonal antibody (*left*) or mouse monoclonal antibody (*right*) and secondary Alexa Fluor conjugated antibody. No significant difference in recombinant protein localization was observed with fluorescence microscopy.

Fig. 2. Immunogold electron microscopy of human recombinant cystatin C, expressed in *Escherichia coli*. Ultrathin cryo sections (70 nm) were labelled with rabbit polyclonal antibody (*left*) or mouse monoclonal antibody (*right*). Gold particles: 10 nm. Polyclonal antibody recognized cystatin C in inclusion bodies and in periplasm, whereas monoclonal antibody recognized predominantly recombinant protein in cell periplasm.

Fig. 3. Quantification of immunogold distributions. Sections were labelled for inhibitor cystatin C with polyclonal antibody (*left*) or monoclonal antibody (*right*). Gold particles were counted on two grids. Distributions of 200 gold particles (+ SD) are shown. Monoclonal antibody recognized predominantly recombinant protein in cell periplasm (72% of a total signal).