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TEM studies of IniA from *Mycobacterium tuberculosis*

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Mycobacterium tuberculosis is one of the main causes of death from a single infectious disease worldwide. A complete understanding of the cellular and molecular mechanisms, in particular structure-function relationships of those proteins that are intimately involved in the pathogenesis, may help design new drugs to treat this infectious bacterium. In the course of studying differential gene expression of *M. tuberculosis*, *iniA, iniB* and *iniC* genes were found to be specifically induced by a broad range of inhibitors of cell wall biosynthesis [1]. Therefore, the characterization of the proteins encoded by *iniABC* genes may aid in the development of new antibiotics.

The iniA gene from *M. tuberculosis* was expressed in *E.coli* with a 6-His tag and the protein purified using a Ni-column. The protein was kept in 100mM Tris-HCl (pH 7.0), 100mM Li_2SO_4 and 1mM DTT. Negatively stained specimens were prepared according to Valentine et al. [2] using an aqueous solution of uranyl acetate (1% w/v, pH 4.25) and examined in a Zeiss 10 C TEM operated at 80 kV. Micrographs were taken at a calibrated magnification (35k x) and digitized with a LeafScan 45 to 5.7 Å / pixel at the specimen level. Particles were selected using the BOXER routine in EMAN [3], and averaged in IMAGIC V [4] after reference-free alignment, multivariate statistical analysis and automatic hierarchical classification. 3-D reconstructions were calculated and refined in EMAN.

Most projections in the electron micrographs show pronounced threefold rotational symmetry while some rectangular structures displaying two parallel lines of higher density could also be observed. The class sums after image processing suggest that IniA has trimeric features but also appears as a double-layered structure in a projection that could constitute a side-on view (Fig. 1). The overall dimensions of these particles are approximately 135 Å across and 80 Å in height. Each of the protein deficits present in the face-on view measures approximately 30 Å in diameter. In Fig. 2, surface-rendered 3-D reconstructions are presented assuming one axis of threefold rotational symmetry and a molecular mass of 219 kDa (top row) as well as 438 kDa (bottom row). With the molecular mass of an IniA monomer being 73 kDa, this would mean that the structure shown is either a trimer or a hexamer. The missing densities in the 219 kDa rendering and the fact that the 438 kDa reconstruction better reflects the 2-D projections obtained prior to imposing symmetry suggest that IniA is a hexamer. It is interesting to note that in addition to the essentially triangular IniA, a hexameric ring form was observed (Fig. 3). These two forms could constitute different configurational states of IniA.

Furthermore, in the presence high salt concentrations (3M sodium formate, 2% w/v phosphotungstic acid, pH 6.5), small IniA crystals were obtained in which the protein appears to have assumed a tetrameric state (Fig. 4).

References
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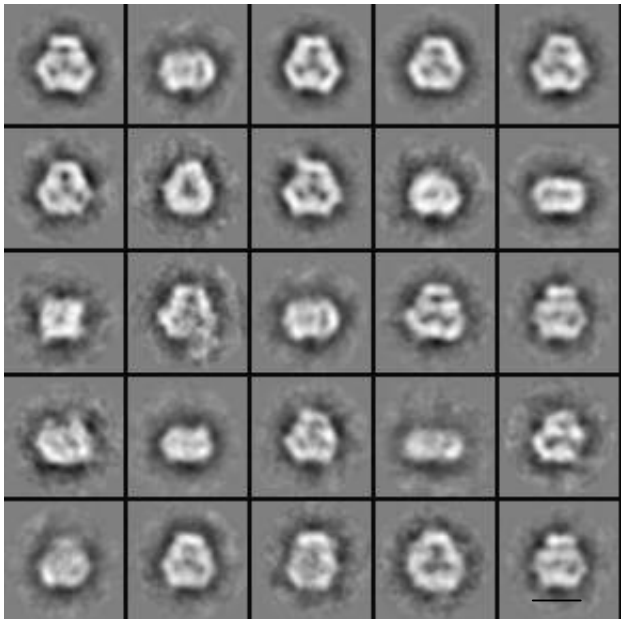

Fig. 1 Class sums of iniA particles.
All scale bars correspond to 10nm.

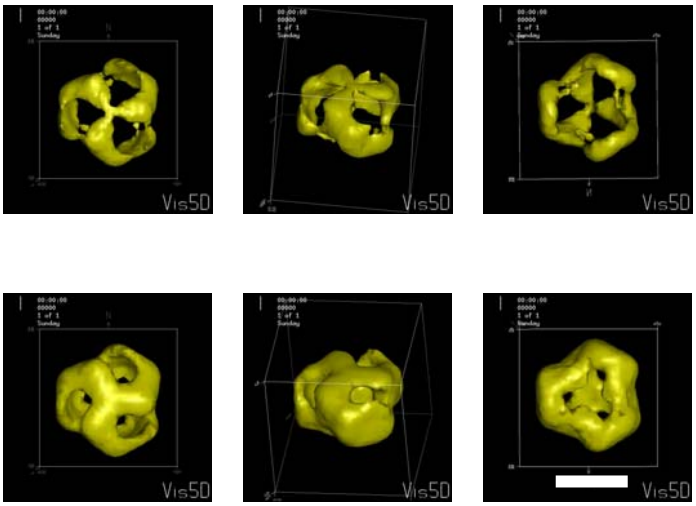

Fig. 2 3-D reconstructions using 210 kDa (top row) and 438 kDa (bottom row) thresholds.

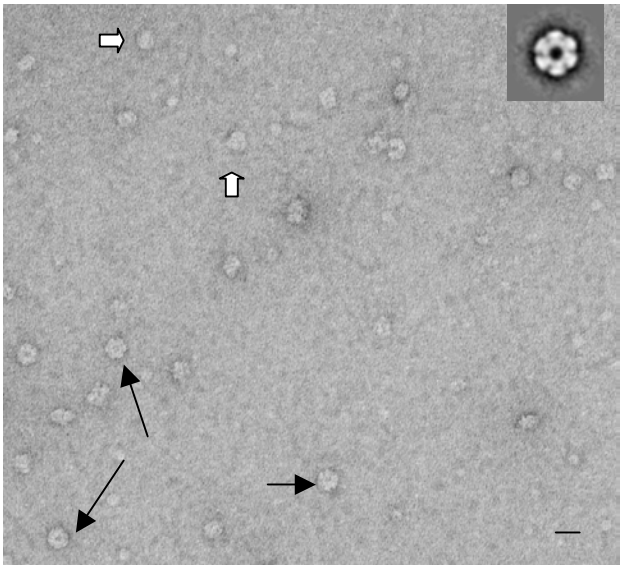

Fig. 3 Triangular(⇧) and hexameric ring forms (→) of IniA.

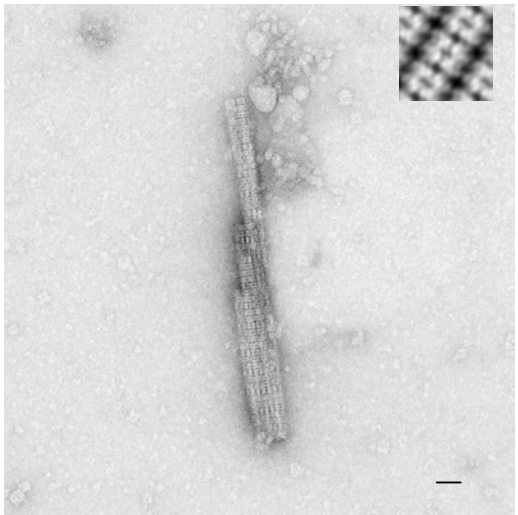

Fig. 4 Negatively stained crystal of IniA. Inset: Projection map calculated in p2.

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