Site-specific mutations of FtsZ - effects on GTPase and in vitro assembly

ABSTRACT

Several mutants with greatly reduced GTPase could still complement ftsZ84, suggesting that the high level of GTPase observed in vitro is not essential for in vivo function. All of the lateral mutants failed to complement ftsZ84, which suggests that these surfaces of the protofilaments are important for function in cell division. These lateral surfaces may mediate association of FtsZ protofilaments into pairs or small sheets, although their structure is apparently different from the sheets assembled in DEAE dextran or calcium.

INTRODUCTION

Background FtsZ assembles into protofilaments in vitro, and these are thought to form the cytoskeletal framework of the bacterial cell division machine of prokaryotes. The FtsZ protein is homologous to tubulin, and the orientation of FtsZ subunits in the protofilament can be deduced by comparing the atomic structures of tubulin and FtsZ. If the tubulin subunit is thought of as a cube, it would have six faces. The top and bottom faces of the subunit, which we call longitudinal, contact subunits above and below it in the protofilament; the right and left faces, which we call lateral, contact subunits in adjacent protofilaments; and the front and back faces correspond to the outside and inside of the microtubule. Since the FtsZ protofilament appears to be a homolog of the tubulin protofilament we will use these same designations for FtsZ. To identify amino acids critical for FtsZ function, we constructed 16 mutations, mostly changing conserved aspartate and glutamate residues to alanine. Initially we selected amino acids for mutation based on conservation across different species, but after the atomic structure of FtsZ was determined we designed other mutations to target specific structural questions. The main focus of the present study was to characterize the in vitro assembly and GTPase of the mutant proteins, but we also did a preliminary survey of their in vivo function by testing their ability to complement the temperature sensitive mutant ftsZ84. More than a dozen mutations of FtsZ have been characterized in previous studies. We include these previously characterized mutations in our analysis of the structurally important side chains of FtsZ.

CONCLUSION

Conclusions We have tested 16 site-directed mutants of E. coli FtsZ for assembly and GTPase activity in vitro, and for whether they can function in vivo to complement ftsZ84. Mutations on the front and back of the FtsZ protofilament were mostly benign: they had normal in vitro assembly, and could complement ftsZ84. Several of these, however, had significantly reduced in vitro GTPase, demonstrating that the high level of GTPase is not essential for assembly or function in vivo. Another class of mutations altered residues contacting the GTP. These could all assemble in DEAE dextran, although with some abnormalities, and they had < 10% of the wild type GTPase. These mutants failed to complement ftsZ84 when expressed on pBS58. A third class of mutations mapped to the sides of protofilaments. These lateral mutations did not interfere with GTPase nor with assembly of protofilaments, and surprisingly showed mostly normal assembly in DEAE dextran. We conclude that these lateral surfaces are not involved in assembly of the DEAE dextran polymers. However, four mutations on the right side and two on the left failed to give clones that complement ftsZ84, suggesting that these lateral surfaces are important for

the function of FtsZ in cell division. These lateral surfaces may mediate association of FtsZ protofilaments into pairs or small sheets, although with a structure different from the in vitro polymers stabilized by DEAE dextran.