Supporting information for Shan and Walter (2003) *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas. 0737693100

**Supporting Text**

A potential caveat of the experiment described in Fig. 2 is that nucleotide specificity may be present in free FtsY but was not observed because the low basal GTPase and xanthosine 5'-triphosphate (XTPase) activity of FtsY is masked by a contaminant nucleotide hydrolase. However, the following observations strongly argue against this possibility. First, FtsY was extensively purified over three columns (1, 2), and the GTPase activity coprofiles with FtsY over the last column (data not shown). Second, the inhibition constant obtained for several nucleotides [GDP, xanthosine 5'-diphosphate (XDP), and GppNHp] are the same, within error, for both the basal GTPase and XTPase reactions, suggesting that the basal GTPase and XTPase activities arise from the same protein active site. Third, the GTP affinity for FtsY determined from the FtsY concentration dependence of the basal GTPase reaction (see text) is the same, within error, as that determined by using inhibition methods or that measured fluorescently (3).

Finally, we determined and compared the inhibition constant of XDP for the basal and signal-recognition particle (SRP)-stimulated XTPase activities of FtsY(D449N). If XDP inhibits the stimulated XTPase reaction with a different inhibition constant than the basal XTPase reaction, then the observed basal XTPase activity must arise from a protein other than FtsY. In contrast, if the same XDP inhibition constant was observed, then it is highly likely that the basal activity also arises from FtsY.

To this end, we determined the rate of the basal and SRP-stimulated XTP hydrolysis reactions at varying XDP concentrations (Fig. 7). To ensure that the XDP affinity for free FtsY(D449N) was measured even in the SRP-stimulated reaction, the following conditions were used: (*i*) A subsaturating concentration of SRP (1 μM; this is within the linear range of the SRP concentration dependence in Fig. 3) was used to ensure that FtsY(D449N) is predominantly in its free form without SRP bound; (*ii*) a subsaturating concentration of FtsY(D449N) (0.2 μM) with respect to its XTP dissociation constant [$K_d^{XTP}$ = 260 μM (Table 2)] was used to ensure that FtsY(D449N) is predominantly in its free form without XTP bound. The XDP inhibition curves for the stimulated XTPase reaction is the same, within error, as that for the basal XTPase reaction (Fig. 7, filled and open circles), giving XDP inhibition constants of 2.1 and 2.2 μM for the basal and SRP-stimulated XTPase reaction, respectively. These XDP inhibition constants are much lower than that for SRP (22 μM; S.S. and P.W., unpublished results), indicating that the inhibition is not due to XDP binding to the GTPase site in SRP. Thus put together, the data provide strong support for the notion that both the basal and stimulated XTPase activities arise from the same active site, that of FtsY(D449N).

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