LEGENDS TO THE SUPPLEMENTAL FIGURES

Fig. S1. Stability of the translocation substrate. A proOmpA (pOA) derivative with cysteines at positions 164 and 202 was translocated into urea-washed inverted membrane vesicles (IMVs) under oxidative conditions for 15 min at 37°C. After formation of a translocation intermediate (lane 2), the sample in lane 7 was further incubated with DTT and ATP (chase). To determine backsliding of the intermediate, the samples in lanes 3-6 were depleted of ATP by addition of hexokinase and glucose (HK/Gl), placed at 37°C, and aliquots were taken at the times indicated. The sample in lane 1 received DTT from the onset of translocation. The sample in lane 8 received HK/Gl at the beginning of the translocation reaction. In sample 9, Triton-X 100 (TX-100) was present during proteolysis. All samples were treated with proteinase K (PK). To ascertain that the substrate remained stable over the time course of the experiment, aliquots of samples 1-9 were analyzed without treatement with proteinase K (lanes 10-18). The samples were separated by SDS PAGE and analyzed by autoradiography.

Fig. S2. Forward translocation into inverted membrane vesicles (IMVs) in the presence or absence of a disruptor of the membrane potential. (A) A translocation intermediate of proOmpA (pOA) with cysteines at positions 175 and 202 was generated with IMVs as in Figure 1A, except that the uncoupler CCCP was added, and an aliquot was immediately digested with proteinase K (PK) (lane 2). 10 mM DTT and excess unlabeled pOA were added, and samples were taken at the times indicated and digested with PK (lanes 3-7). Translocation in the presence of DTT (lane 1) or hexokinase glucose (lane 9) are shown. An aliquot of sample 2 was proteolyzed in the presence of Triton-X 100 (TX-100) (lane 8). The sample in lane 10 received excess unlabeled pOA at the beginning of the translocation reaction. Lanes 11-20 are the same experiment, except that CCCP was omitted. (B) Quantification of translocation assays performed as in (A). For the disappearance of the intermediate, the intensities were normalized to that observed at t=0. For the appearance of the full length species, intensities were normalized with respect to the plateau level at t=120 sec.

Figure S1

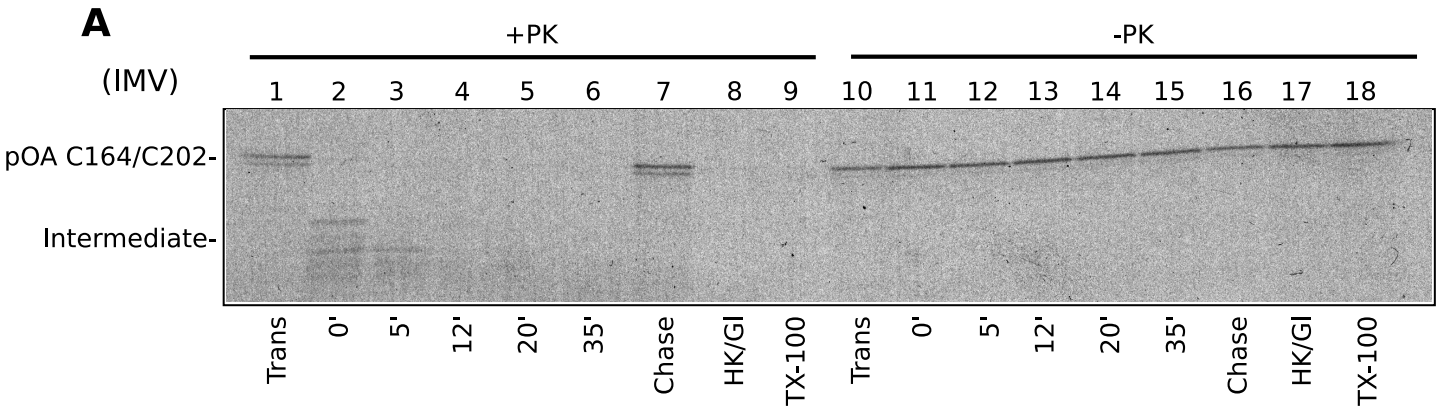
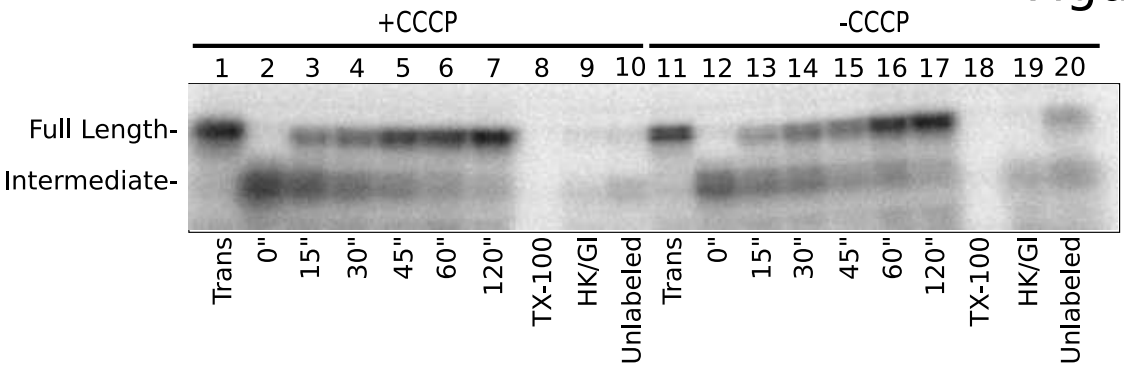
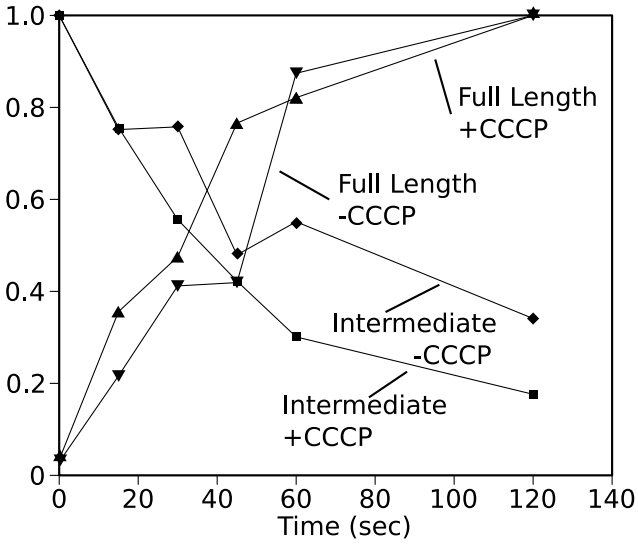

A
(IMV)
+PK
-PK
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
pOA C164/C202-
Intermediate-
Trans
0'
5'
12'
20'
35'
Chase
HK/GI
TX-100
Trans
0'
5'
12'
20'
35'
Chase
HK/GI
TX-100

Figure S2

A


+CCCP
-CCCP
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Full Length-
Intermediate-
Trans
0"
15"
30"
45"
60"
120"
TX-100
HK/Gl
Unlabeled
Trans
0"
15"
30"
45"
60"
120"
TX-100
HK/Gl
Unlabeled

B


1.0
0.8
0.6
0.4
0.2
0
0
20
40
60
80
100
120
140
Time (sec)
Full Length
+CCCP
Full Length
-CCCP
Intermediate
-CCCP
Intermediate
+CCCP