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Interplay with a co-translational chaperone enhances specificity of mammalian SRP pathway

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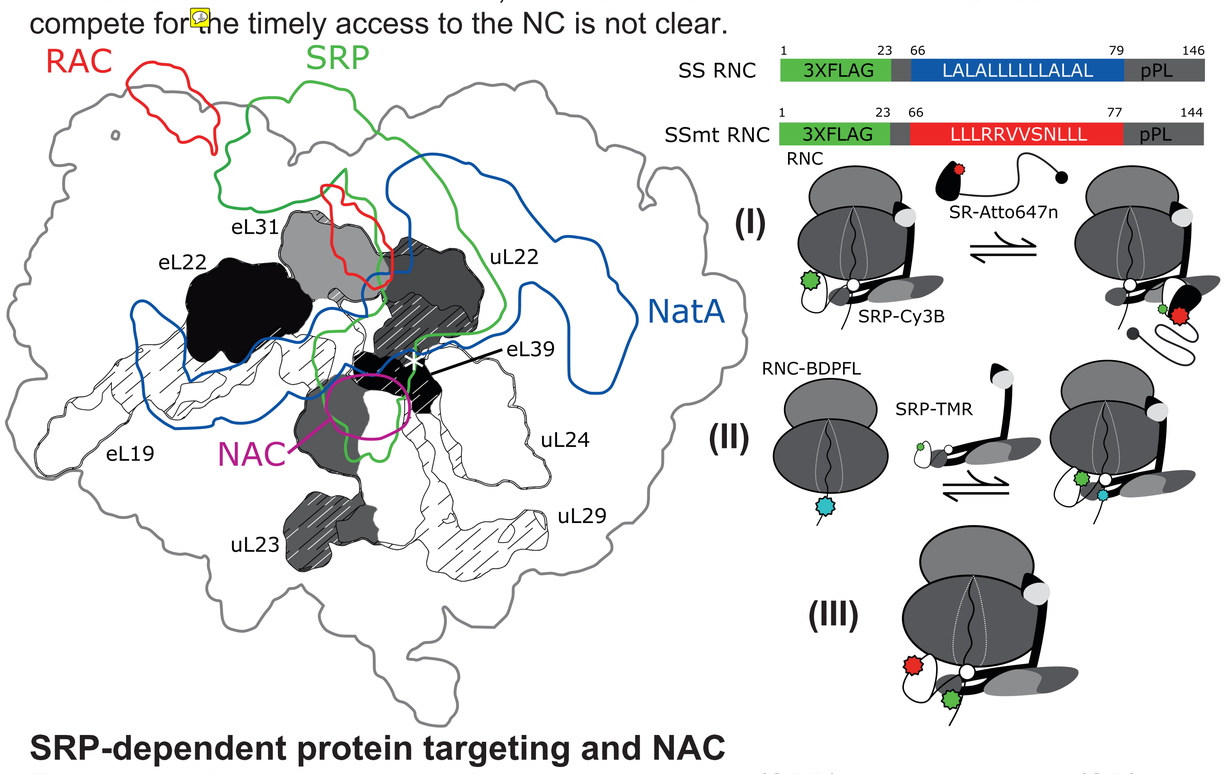
Introduction

Crowded environment at the ribosome tunnel exit

Nascent chain (NC) engages with multiple ribosome-associated protein biogenesis factors (RPBs) cotranslationally at the ribosome exit for correct targeting, modification or folding. By overlaying several available structures of RPB bound ribosome from eukaryotes (1-4), it is evident that multiple RPBs have overlapping binding sites near the ribosome tunnel exit. However, the mechanism of how these RPBs coordinate or compete for the timely access to the NC is not clear.



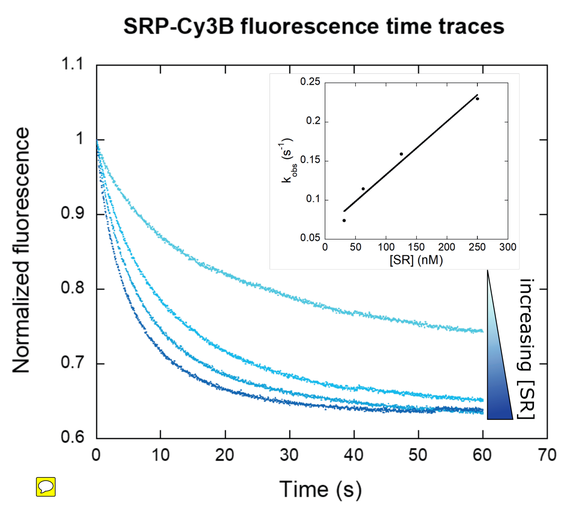
SRP-dependent protein targeting and NAC

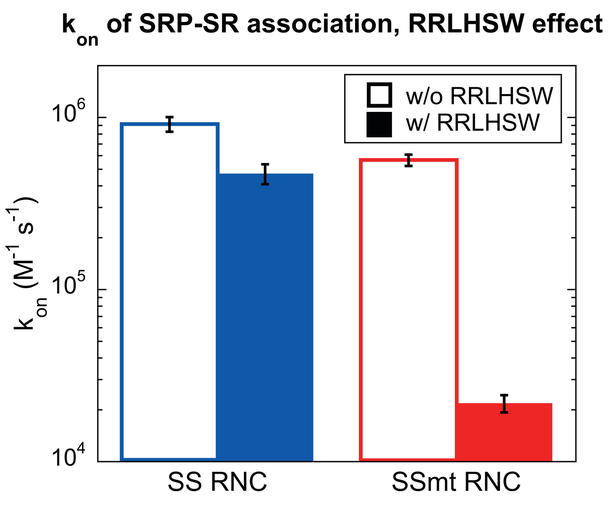
The universally conserved signal recognition particle (SRP) and its receptor (SR) co-translationally target one-third of the proteome to the destined membrane for secretion or membrane integration. Unlike its prokaryotic counterpart, the mammalian SRP has been previously shown to be marginally specific to a signal sequence (SS) (5). On the other hand, nascent polypeptide-associated complex (NAC), previously identified as a cotranslational chaperone, has been shown to enhance the specificity of cotranslational targeting. However, it is not clear whether NAC directly interacts with SRP to change the targeting activity and what the mechanism is.

Results

Reconstituted cytosolic RPBs enhance SRP specificity (I)

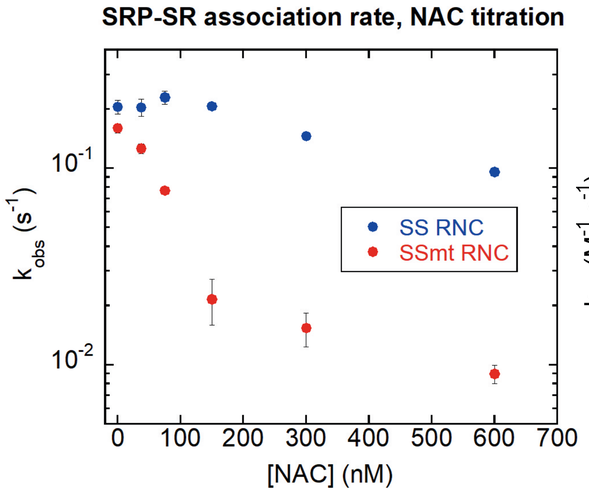
To understand how RPBs affect the specificity of SRP pathway, we used FRET based SRP-SR association assay to test the activation of SRP by RNCs with strong or mutated SS. The cytosolic RPBs were purified from rabbit reticulocyte lysate (RRL) by high salt buffer wash of ribosome (HSW). RNCs were synthesized by in vitro translation in RRL. Association rates of purified SRP and SR shows only two-fold difference between SS and SSmt RNCs, while RRLHSW enhances the difference to nearly 25-fold, suggesting that cytosolic RPBs can increase specificity of SRP-SR.

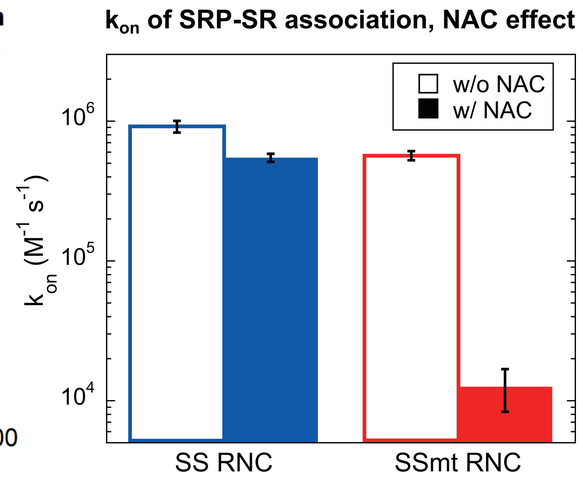




NAC alone can improve the specificity of SRP (I)

To pinpoint the effect of NAC on the activity of SRP, we recombinantly expressed and purified NAC from *E. coli* and supplemented NAC into SRP-SR association assay. SRP-SR association rate drops sharply once the NAC is titrated to the concentration of SSmt RNC, suggesting that NAC bound SSmt RNC is incompetent in SRP activation. On the other hand, SS RNC maintains almost the same level of SRP-SR association rate even at super-stoichiometric concentrations. We further determined the k_{on} of SRP-SR complex at a saturating NAC concentration. We found that NAC increases the specificity of SRP-SR system from 2-fold to roughly 40-fold. These data indicate that NAC binding to RNCs without strong SS can significantly slow down SRP-SR association and accounts for most of the specificity enhancement by reconstituted cytosolic RPBs from RRLHSW.

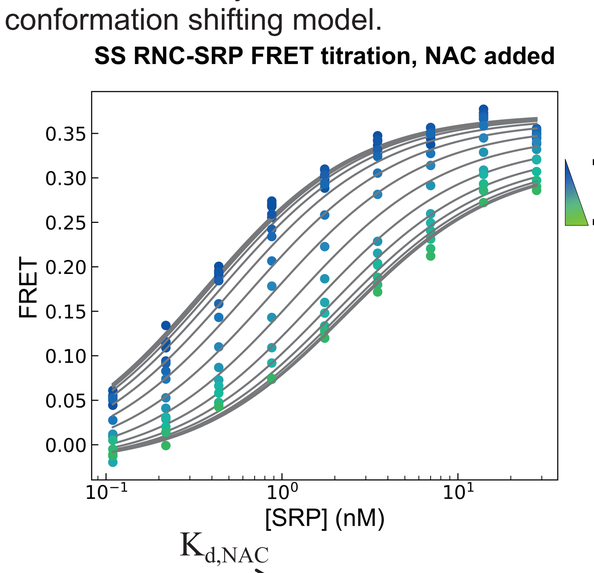


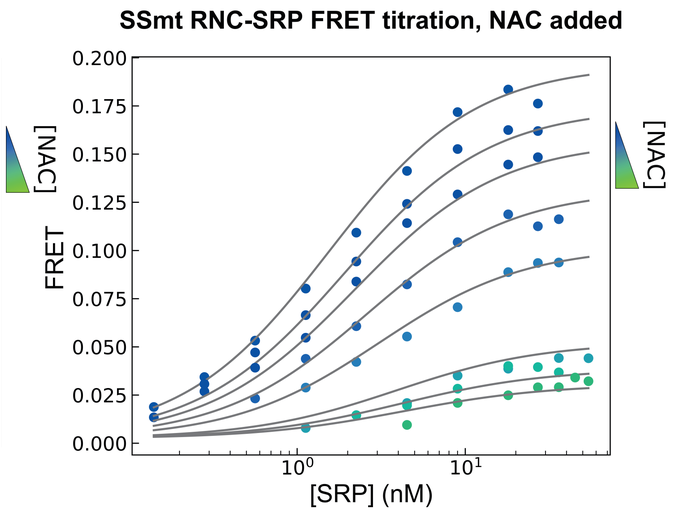


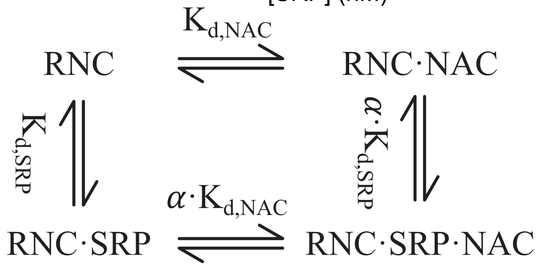
NAC changes the conformation of SRP on RNC (II)

Two potential models can explain previous data: (1) NAC selectively binds to SSmt RNC and is in direct competition with SRP binding; (2) NAC and SRP can both bind to SS or SSmt RNC at the same time, but NAC selectively shifts the conformation of SRP on SSmt RNC toward an inactive one.

We first determined the binding state of NAC and SRP on RNCs by designing a FRET pair probe on SRP and RNC. The FRET signal between SRP and RNCs allowed us to determine the effect of NAC on SRP-RNC binding by FRET titration at varying concentrations of NAC. The results were pooled and fitted to an anti-cooperative model. We found that NAC can bind to RNCs at low nM K_d and anti-cooperativity of around 6.6 and 2.8 for SS RNC and SSmt RNC, respectively. Although the binding of SRP is weakened upon NAC binding, the low anti-cooperativity means that effective K_d of SRP when NAC is also bound is roughly 3-4 nM, well saturated throughout the SRP-SR association assay. This is inconsistent with the direct competition model and favors the conformation shifting model.



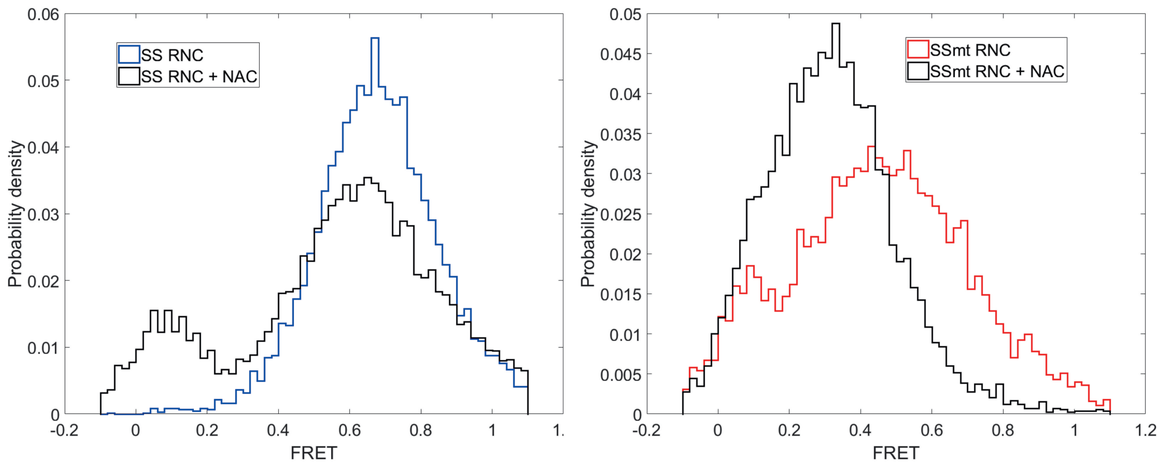




	SS RNC	SSmt RNC
$K_{d,SRP}$ (nM)	0.36±0.01	1.5±0.1
$K_{d,NAC}$ (nM)	1.4±0.1	1.2±0.2
α	6.6±0.4	2.8±0.5

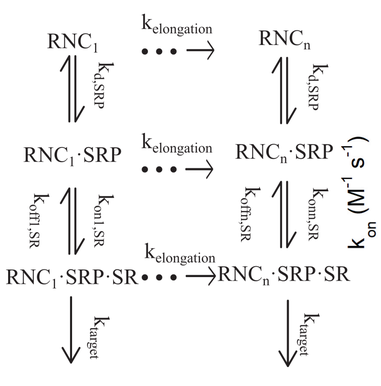
Single molecule characterization of SRP conformation (III)

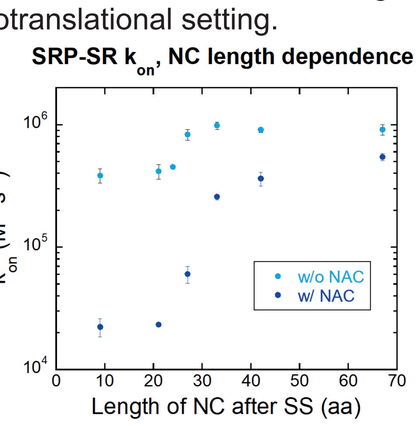
To directly observe the shift in conformation upon NAC binding, we used previously characterized FRET pair on SRP probing the distance between GTPase domain and the NC to measure the distribution of SRP in different conformations in a single molecule setup (5). When bound to SS RNCs, SRP is mostly in the high FRET state and does not shift significantly when NAC is added. On the other hand, SRP bound to SSmt RNC starts with mixed states ranging from low to high FRET but loses all high FRET state upon NAC binding. The observed trend suggests that SRP-SR association is the fastest with SRP in the high FRET state, which NAC specifically inhibits for SSmt RNC-bound SRP.

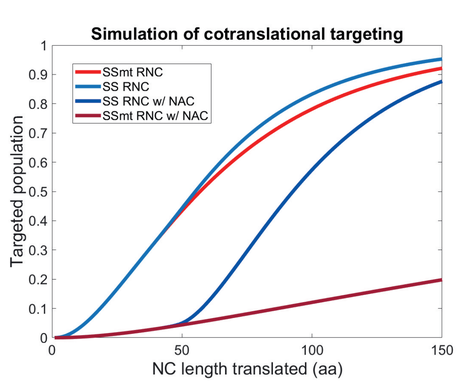


NAC improves cotranslational targeting specificity (I)

Targeting of RNC by SRP is a cotranslational process that necessarily compete with the NC elongation. We assessed the specificity of SRP pathway in the context of ongoing translation with or without NAC by measuring SRP-SR association rate at increasing NC length and numerical kinetic simulation. Modeled with in vivo elongation rate and concentrations of SRP, SR and NAC, we found significantly increased specificity when NAC is considered in a cotranslational setting.







Conclusions

In this study, we used bulk and single molecule FRET assays to show that (1) SRP and NAC co-bind on RNC anti-cooperatively; (2) NAC specifically inhibits SRP-SR association for RNC with weak SS; (3) The slowdown is caused by the shift in SRP conformation; (4)The slowdown is necessary to maintain the specificity of cotranslational targeting.

Acknowledgements

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References

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