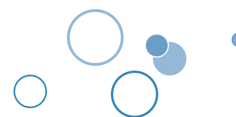
Ensuring that Proteins Reach Their Proper Destinations

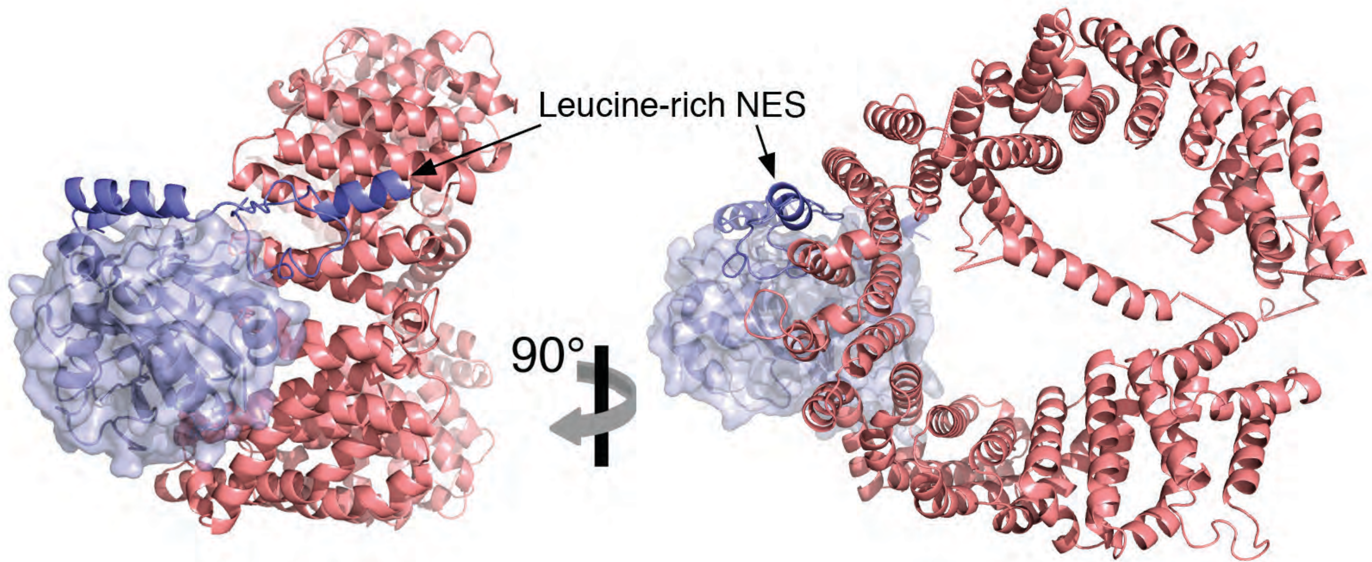


IN SHORT > In eukaryotes (organisms with a cell nucleus), proteins are made by transcribing DNA into messenger RNA (mRNA), which is then translated to build proteins from amino acids. DNA is in the nucleus, and protein synthesis occurs outside the nucleus in the cytoplasm. However, many newly made proteins "work" in the nucleus and have to be transported there after they are made. The details of this transportation process are a mystery investigated by researchers from the University of Texas Southwestern Medical Center at Dallas, using the SBC-CAT 19-ID beamline at the APS.

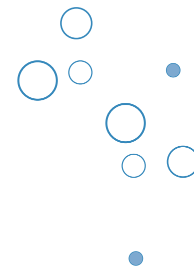
MORE > Separation of nuclear activities from cytoplasmic activities is tightly regulated, so a complex but efficient system of moving cellular components into and out of the nucleus has evolved. This transport process involves a postal-like system wherein proteins contain an amino acid sequence that "tags" them for import into or export out of the nucleus. Special transporters recognize these nuclear localization signals (NLS) or leucine-rich nuclear export signals (NES), and guide them through pores in the nuclear membrane to their destinations. One of these transporter proteins is called CRM1 (exportin-1) and it is responsible for the transport of hundreds of proteins out of the nucleus. One of the CRM1 cargo proteins is called snurportin (SNUPN), which moves ribonucleoproteins into the nucleus and then must be recycled back out. Although amino acid sequence "tags" have been identified for many proteins that are imported into and out of the nucleus, many other proteins with similar sequences are, in fact, not transported. This suggests that these signals are not the only determinant for this process. This study has provided some answers to this mystery: The structure of CRM1 in complex with SNUPN at 2.9-Å resolution, solved by multiwave-length anomalous dispersion at the 19-ID beamline, has shown how CRM1 recognizes the SNUPN leucine-rich NES and an additional binding surface between these proteins, and has revealed how the drug Leptomycin B inhibits nuclear export by CRM1.

The first mystery solved by elucidation of the structure was the location of the leucine-rich NES on SNUPN. Investigators had previously reported that SNUPN didn't have a leucine-rich NES, but is an atypical cargo that bound CRM1 using a 300-amino acid domain. However, when the researchers in this study looked at the structure, they observed that a sequence in the N-terminus of SNUPN responsible for CRM1 recognition did actually contain the consensus for a leucine-rich NES. The leucine-rich NES nestles into a groove between two α-helices in ring-shaped CRM1 that provides a hydrophobic environment for the interaction (Fig. 1). Alteration of hydrophobic amino acids in the leucine-rich NES or the hydrophobic NES-binding groove of CRM1 interrupted the binding of CRM1 to SNUPN, proving that the N-terminus of SNUPN is a true NES and its binding site on CRM1 is the bona fide NES-binding site.

Leucine-rich NESs are known to be short stretches of hydrophobic amino acids interspersed with other amino acids. The interactions mediated by these regions are usually weak and these sequences are often found in proteins that are not exported from the nucleus. This led to the notion that there must be other factors involved in the specificity of nuclear export recognition. As predicted, the CRM1/ SNUPN structure shows that SNUPN contains a basic region that interacts with a complementary acidic surface on CRM1 adjacent to where it binds the SNUPN leucine-rich NES (Fig. 1). This interaction stabilizes the interaction with SNUPN and is likely to be present in other CRM1 cargoes. Interestingly, this model of bipartite binding of nuclear export receptor to its cargo is similar to one observed earlier for nuclear import processes and



^ *Fig. 1 Overall structure of the CRM1–SNUPN complex. CRM1 is shown in pink and SNUPN is shown in blue. SNUPN binds CRM1 through its leucine-rich NES (labeled) and its basic nucleotide binding domain (shown as surface representation).*



suggests that these two processes are more similar than previously appreciated.

Finally, the CRM1/SNUPN structure was used to answer another outstanding question. Leptomycin B is an anti-fungal anti-bacterial agent that is known to inhibit nuclear export by binding to cysteine 528 of CRM1. In the structure, cysteine 528 sits in the binding groove for the SNUPN leucine-rich NES, showing clearly why Leptomycin B inhibits nuclear export by CRM1. This is important information because Leptomycin B, in addition to its uses as an anti-fungal and anti-bacterial agent, has shown promise as an anti-cancer agent. Knowledge of its mode of action could inform drug design efforts aimed at improving the efficacy of CRM1 inhibitors in medicine.

— *Sandy Field*

See > Xiuhua Dong, Anindita Biswas, Katherine E. Süel, Laurie K. Jackson, Rita Martinez, Hongmei Gu, and Yuh Min Chook*, "Structural basis for leucine-rich nuclear export signal recognition by CRM1," Nature **458**, 1136 (30 April 2009). DOI:10.1038/nature07975

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> 19-ID • SBC-CAT • Life science • Anomalous diffraction (MAD/SAD), subatomic (<0.85 Å) resolution, ultra-low-temperature (15K) • 3.3-cm Undulator A • Accepting general users