

Leptomycin B: A Powerful Antibiotic Tool for Studying Nuclear Transport

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Introduction

Leptomycin B (LMB) is an antibiotic with anti-fungal and anti-tumor activity that was first discovered and purified from the fermentation broth and mycelia of *Streptomyces*.¹⁻⁴ LMB (C₃₃H₄₈O₆, MW 540) is an unsaturated, branched chain fatty acid with a terminal lactone ring (Figure 1).² Recently, this antibiotic has become an important tool for studying nuclear localization and trafficking in eukaryotic cells, due to specific inhibition of the CRM1/exportin1 nuclear export pathway.

Discussion

Proteins and other macromolecules constantly move into and out of eukaryotic cell nuclei. Bi-directional nuclear transport is a regulated, signal-mediated process that occurs through specific proteinaceous structures, the nuclear pore complexes, which span the nuclear envelope.⁵⁻⁸ In the last decade, there has been much progress in understanding the composition and structure of the nuclear pore complex and the mechanisms of nuclear transport. A number of important discoveries have helped elucidate the protein import process. This includes characterization of the classic nuclear localization signal (NLS), discovery of NLS recognition by the soluble adaptor protein, importin- α , the role of the importin- β receptor subunit for import and nuclear release of cargo protein, and the role of Ran-GTP in cargo discharge and transport directionality.⁷⁻¹⁰ The importin- β protein is one member of a conserved family of transport receptors, also termed

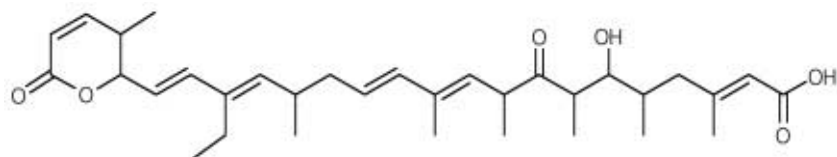


Figure 1. Chemical structure of leptomycin B (LMB).

karyopherins. Each member recognizes a distinct NLS. Karyopherins can also bind the small GTP-binding protein, Ran, and nuclear pore complex proteins (nucleoporins).

More recently, details of the related mechanism for nuclear export have emerged.⁷⁻¹⁰ Much of the early progress has come from studies of the HIV Rev protein and the cellular PKI protein (protein kinase A inhibitor). These studies led to the discovery and characterization of the leucine-rich nuclear export signal (NES) and the CRM1/exportin1 pathway of nuclear export. CRM1 is a karyopherin specific for nuclear export (exportin). A number of reports have

now established that the CRM1 protein directly binds proteins that contain a leucine-rich NES.¹¹⁻¹⁴ In addition, the export mechanism appears to involve CRM1 binding to both Ran-GTP and nucleoporins.¹⁵

The stability of the karyopherin/cargo protein interaction is modulated by the nucleotide-bound state of Ran, which modulates import and export differentially.^{9,10} Interactions of importins with their cargo proteins are disrupted by Ran-GTP, which is maintained at high levels in the nucleus by the presence of its guanine nucleotide exchange factor, Ran-GEF, specifically localized in the nuclear compartment. This results in the rapid discharge of the cargo when the complex reaches the nucleus. In contrast, the interactions of exportins with their respective cargo proteins are stabilized by Ran-GTP binding. These interactions are disrupted upon Ran-GTP hydrolysis, which occurs rapidly in the cytoplasm, due to the presence of high levels of the Ran GTPase activating protein, Ran-GAP, in the cytoplasmic compartment. Thus, the compartment-specific localization of the regulators of the Ran nucleotide-bound state is responsible for the Ran-GTP gradient across the nuclear membrane. The Ran-GTP gradient effectively determines the directionality of nuclear transport by regulating cargo binding and release.

Tools available for perturbing nuclear transport are still somewhat limited and include genetic loss of function mutants or dominant mutants in karyopherins or the Ran GTPase regulation pathway. While there are no specific drugs for inhibiting nuclear import pathways, there is a drug that inhibits nuclear export. It has recently been shown that the anti-fungal antibiotic, Leptomycin B, specifically and potently inhibits the CRM1/exportin 1 pathway of nuclear export by directly binding the CRM1 protein.

Leptomycin B has been an important tool in the elucidation of the role of CRM1/exportin 1 in the export process. The inhibition of growth of fission yeast and mammalian cells by LMB⁴ was shown to be due to the inhibitory effect of LMB on CRM1-mediated processes.¹⁶

Later studies on HIV-Rev protein export determined that LMB is an inhibitor of nuclear export.¹⁷ It now has been demonstrated clearly that the cellular effects of LMB are due to inhibition of nuclear export as a consequence of LMB binding covalently to the CRM1

protein. Binding of LMB to CRM1 occurs in its conserved central region at a critical cysteine residue and prevents formation of the complex between CRM1 and the NES of cargo proteins.^{18,19} Unlike fission yeast and mammalian cells, *Saccharomyces cerevisiae* does not show toxic effects of LMB treatment. However, when a single threonine in the conserved central region of the *S. cerevisiae* CRM1 protein (Thr539) is changed to cysteine, the resulting strain becomes fully sensitive to LMB.²⁰ Thus, CRM1 structure and function appears to be conserved throughout eukaryotes.

It is becoming increasingly clear to cell biologists that the steady state localization of proteins does not always reflect their biologically important, functional sites of action. For example, steady state localization of a protein in the cytoplasm may not allow its dynamics in and out of the nucleus to be detected. However, the use of Leptomycin B as a tool to block CRM1-mediated nuclear export results in the accumulation of NES-containing proteins in the nucleus and allows their detection in the nuclear compartment (Figure 2).

Summary

Understanding the mode of action for Leptomycin B inhibition of the CRM1/exportin 1 nuclear export pathway has significantly increased the utility of LMB for cell biology studies on nuclear transport and localization. Recently, Leptomycin B has been used in many systems to demonstrate nuclear localization and CRM1 pathway-dependent export of a number of proteins, including actin, transcription factors, kinases and cell cycle regulators (Figure 3).²¹⁻²⁷ The creation of a LMB sensitive strain of *S. cerevisiae* has further increased the utility of this antibiotic.²⁰ Usage of Leptomycin B for study of nuclear localization and export should continue to increase in the future, as more purified and reliable supplies become commonly available to researchers.

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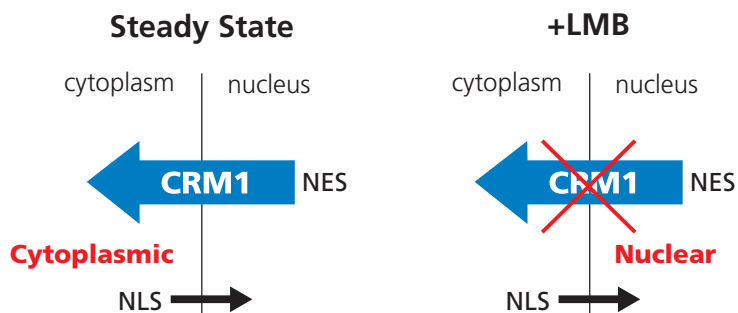


Figure 2. Leptomycin B specifically blocks NES-dependent nuclear transport through the CRM1 nuclear export pathway. Biologically relevant nuclear localization of proteins is not always obvious. Steady state localization of a protein can appear predominantly cytoplasmic, if the equilibrium of bi-directional nuclear transport favors nuclear export. However, blockage of CRM1-mediated nuclear export by LMB results in accumulation and detection of proteins containing nuclear export sequences (NES) in the nucleus.

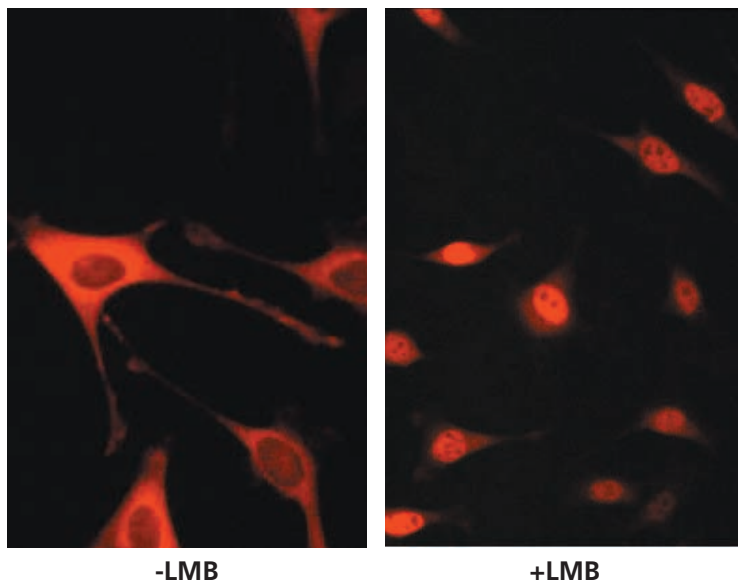


Figure 3. Nuclear localization of cyclin B1 revealed by treatment of cells with Leptomycin B. HeLa cells, synchronized at the G1/S border by a double thymidine block, were released from the block and either left untreated (-LMB) or incubated with 20 nM Leptomycin B (+LMB) for 2.5 hr prior to fixation. Consequently, cells were fixed and subjected to indirect immunofluorescent staining with a monoclonal antibody specific for cyclin B1 and an indocarbocyanine (Cy3)-conjugated donkey anti-mouse secondary antibody. Staining was visualized by conventional fluorescence microscopy. In untreated cells, cyclin B1 is predominantly localized in the cytoplasm. In LMB treated cells, nuclear export is specifically blocked and cyclin B1 accumulates in the nucleus. [From H. Pwinica-Worms, Dept. Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO.]

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ORDERING INFORMATION

Product Code	Product Name	Unit	Price
L 2913	Leptomycin B >95% by HPLC 0.5 μ g in 100 μ l 70% methanol	0.5 μ g	\$300.00