

# Nuclear and nucleolar localization sequences

## Introduction

Proteins larger than ~50kDa are too large to passively diffuse from the cytoplasm to the nucleus and must be actively transported across the NPC. These proteins must contain a nuclear localization sequence/signal (NLS) in order to be recognized by the alpha subunit of importin. When the cargo protein binds to alpha importin, alpha importin binds to beta importin, and beta importin is recognized by the FG repeat domains on the cytoplasmic filaments of the NPC.

Once in the nucleoplasm, some proteins move on to localize to the nucleolus. Nucleolar proteins are involved in cell cycle, ribosome biogenesis, and rRNA production. In nucleolar proteins, the NLS usually functions as a NoLS as well.

## Nuclear localization sequences/signals (NLSs):

- NLS must be present to bind  $\alpha$ -importin
- NLS-containing protein must then cross NPC to enter nucleus
- Conserved sequence includes high proportion of basic residues, especially arginine (R) and lysine (K)
- Majority ~4 – 20 residues in length
- Can be monopartite (classic) or bipartite

Monopartite:

Conserved residues are consecutive (basic residues shown in red)

Ex. PKKKKRVG  
GRKRKRRT  
ARKRKRT  
NRRQKGRS  
ECRRKKKE

Bipartite:

Conserved residues are not consecutive – divided into two groups divided by a spacer, usually (K/R)(K/R)xxxxxxxxxxxx(basic residues; starts with either KK or KR, then any 10-12 residues, then a monopartite NLS follows).

Ex. KKWTPYNSCDLIRRRK

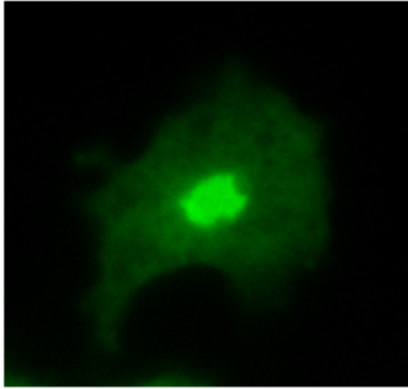
## **Examples of proteins containing NLSs:**

Histones

Transcription factors

Nucleoplasmins

Heat shock proteins (Hsps)



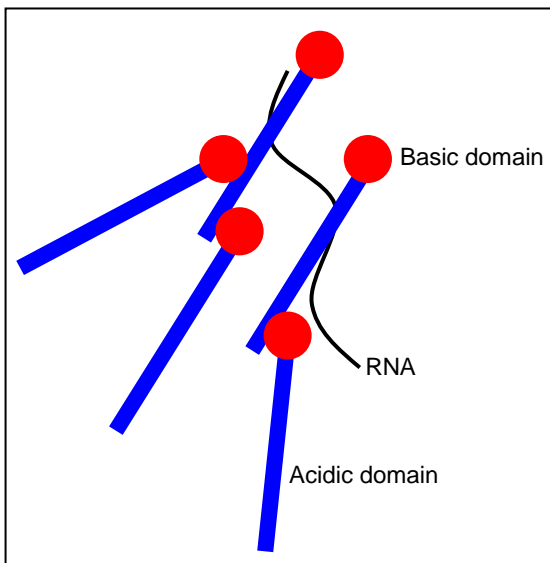
### **Example of nuclear localization**

Nucleomorphin, a nuclear CaM-binding protein in *Dictyostelium* containing multiple monopartite NLSs and a single bipartite NLS. Antibodies against nucleomorphin were produced and immunolocalization was performed.

### **Nucleolar localization sequences (NoLSs):**

- Most NLSs act as NoLSs as well
- No barrier to cross between nucleus and nucleolus. Nucleolus is just another area of nucleoplasm
- Nucleolar proteins contain long stretches of acidic residues, often flanked by basic NLSs. These acidic domains act as “magnets” for which other nucleolar proteins, containing basic NLSs, can bind

### **Acidic “magnets” of nucleolus:**

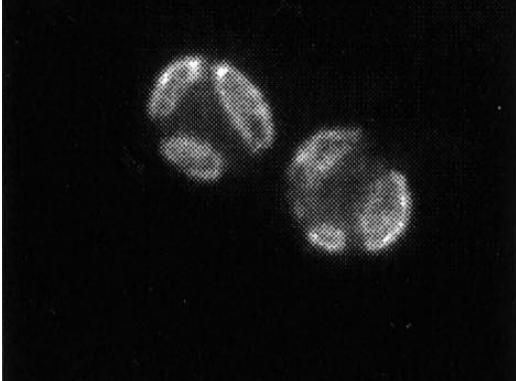


RNA is generally positive/basic. The long acidic domains in nucleolar proteins interact with the positive RNA. These acidic domains can also interact with basic domains of other nucleolar proteins. These interactions thus results in areas of the nucleolus that are both largely positive and negative. What is the significance of this?

**Examples of proteins containing NoLSs:**

Nucleoplasmins

Hsps



**Example of nucleolar localization**

Heat shock protein 32 (Hsp32) is a nucleolar protein also found in *Dictyostelium*. The nucleolus in this organism exists as discrete patches adjacent to the nuclear envelope. Antibodies were produced against Hsp32 and immunolocalization was performed. The cytoplasm in this picture is not visible (Moerman and Klein, 1998).