

# Going in GTP cycles in the nucleolus

Tom Misteli

National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Proteins are directed to cellular compartments by specific localization signals. A GTP-driven cycle has now been identified as a mechanism for protein targeting to the nucleolus. The involvement of a GTP switch suggests that nucleolar localization can be regulated and may be responsive to extracellular stimuli via signaling pathways. The uncovered mechanism also implies that localization is determined by increased retention rather than directed targeting.

A hallmark of mammalian cells is the presence of cellular compartments containing distinct sets of resident proteins. These are directed to their home compartment via short peptide stretches that act as targeting signals. Mechanisms for guiding proteins into the secretory pathway, to cytoplasmic organelles or to the cell nucleus have been extensively characterized. The situation is somewhat different in the nuclear interior. It has been difficult to find strong consensus sequences that direct proteins to one of the many subnuclear domains. Even large-scale proteomic analysis has, for example, not revealed a simple signal that targets proteins to the nucleolus (Andersen et al., 2002; Scherl et al., 2002). Identifying the molecular pathways for localization of proteins to nuclear compartments is a critical task in understanding nuclear function and its regulation. Tsai and McKay report in this issue the first detailed molecular mechanism for targeting of a protein to the nucleolus (Tsai and McKay, 2005).

Nucleolar proteins are increasingly recognized as possible regulators of cell growth and proliferation (Olson, 2004). One such regulator is the nucleostemin protein. Nucleostemin has been reported to be preferentially present in embryonic and adult stem cells of several lineages and to be abruptly down-regulated during differentiation (Tsai and McKay, 2002). The presence of nucleostemin in highly proliferative cell types led to the suggestion that this protein contributes to regulation of growth. In support, misexpression of nucleostemin by overexpression or depletion has antiproliferative effects. Consistent with its possible role in proliferation, nucleostemin appears overexpressed in at least some cancer cell lines (Tsai and McKay, 2002).

Nucleostemin accumulates in the nucleolus. Its NH<sub>2</sub>-terminal basic domain is necessary and sufficient for this localization. In addition, the protein contains two GTP-binding domains, which prompted Tsai and McKay to ask whether these domains contribute to protein localization. Sure enough, mutations in the GTP-binding sites caused decreased binding of

GTP to nucleostemin and displaced the protein from the nucleolus leading to its diffuse nuclear distribution. This observation directly shows that GTP binding is important for nucleolar localization of nucleostemin.

But how does GTP binding lead to nucleolar accumulation? A key observation to resolve this question is the fact that nucleostemin does not statically associate with the nucleolus, despite its steady-state enrichment in the organelle. Like many other proteins with nucleolar residency (Chen and Huang, 2001), nucleostemin rapidly cycles between the nucleolus and the nucleoplasm. Since GTP-binding domains often function as molecular switches, Tsai and McKay hypothesized that GTP binding might modulate the dynamic association of nucleostemin with the nucleolus. Combinatorial deletion analysis and photobleaching microscopy to test the binding dynamics *in vivo* revealed indeed a GTP-driven localization cycle (Tsai and McKay, 2005). This cycle centers around the action of an inhibitory domain that modulates in a GTP-dependent fashion the ability of the basic localization domain to stably interact with nucleolar components (Fig. 1). Binding of GTP allows the basic domain to undergo long-term interactions with nucleolar components, resulting in the accumulation of the protein in the nucleolus. Exchange of GTP in the nucleolus then reverses this stable binding and releases the protein into the nucleoplasm, establishing a GTP-driven cycle. How exactly the inhibitory domain exerts its effect, whether through steric hindrance of the basic domain or via additional nucleoplasmic or nucleolar protein factors, and what nucleostemin binds to in the nucleolus remain to be determined. Answers to these questions will not only reveal a great deal about the localization mechanism, but will likely also have a critical impact on uncovering nucleostemin's role in proliferation.

An important implication from this localization mechanism is that the steady-state localization of nucleostemin in the nucleolus is not due to increased targeting, but is primarily the result of prolonged nucleolar retention. Thus, the "targeting" signal contained in the basic domain is in reality a "retention" signal whose function is to capture the rapidly diffusing protein at a specific spatial location. It seems likely that the principle of retention rather than directed targeting to determine localization is not only valid for nucleostemin, but for many proteins that accumulate in nuclear subcompartments and other cellular locales (Dundr and Misteli, 2002). Such use of retention of diffusing molecules as a mechanism for localization is consistent with a suggested contribution of self-organization to cellular architecture (Misteli, 2001).

Control of a protein's function or localization by a GTP cycle is generally a smoking gun for its regulation by signaling cas-

Correspondence to Tom Misteli: [misteli@mail.nih.gov](mailto:misteli@mail.nih.gov)

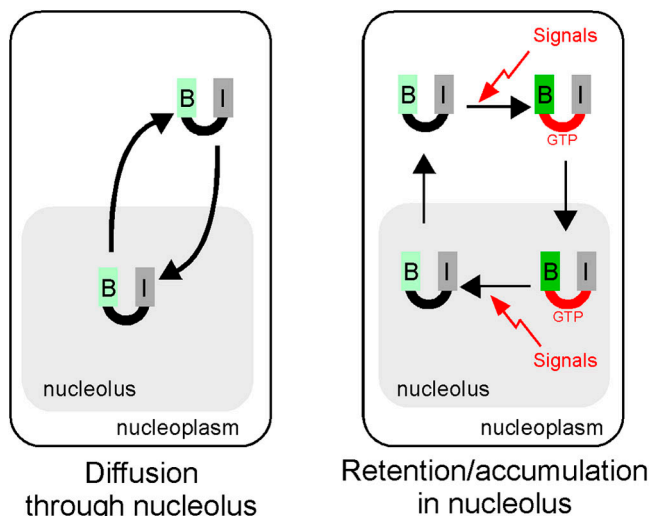


Figure 1. **Nucleolar localization by GTP-dependent retention.** (Left) In the absence of GTP binding, nucleostemin diffuses rapidly through the nucleolus. (Right) Upon binding of GTP, the inhibitory action of the inhibitory domain (I) is relieved. As nucleostemin diffuses through the nucleolus, the basic domain (B) is able to undergo lasting interactions with nucleolar components. Nucleostemin accumulates in the nucleolus due to its prolonged retention. Exchange of GTP reverses the stable binding, and nucleostemin dissociates from the nucleolus. Signaling pathways may affect the availability of the protein for nucleolar import and/or its retention.

acades. Given the dynamic nature of its localization mechanism, changes in the nucleolar concentration of nucleostemin might easily be achieved by either influencing the amount of available nucleostemin that can be imported into the nucleolus or by controlling the retention kinetics. In support, the authors show that lowering intracellular GTP levels by pharmacological agents and withdrawal of cells from the cell cycle results in a decrease in nucleolar retention of nucleostemin via modulation of its dynamic exchange properties. How sensitive nucleostemin localization is to GTP levels and whether changes of the size observed under physiological conditions during signaling events is sufficient to alter the protein's localization remains to be seen. It might be more plausible to assume that GTP/GDP exchange factors in the nucleoplasm and the nucleolus are responsible for transmitting signals to nucleostemin, although the identity of the exchange factors for nucleostemin is not known. Regardless, the potential connections between nucleolar localization and signaling are tantalizing given the protein's link to proliferation control.

The dynamic accumulation of nucleostemin is reminiscent to that of other regulators of proliferation and cell cycle control. Sequestration into the nucleolus of MDM2, an E3 ubiquitin ligase that marks p53 for destruction, results in reduced rates of p53 degradation and has been suggested to contribute to p53 regulation (Olson, 2004). Similarly, in *S. cerevisiae* the cell cycle regulator *cdc14* appears to be stored in the nucleolus until it becomes released before anaphase to initiate a sequence of events to promote progression through late M-phase and exit from mitosis (Azzam et al., 2004). Interestingly, in all those cases, the site of action of these nucleolarly sequestered proteins is in the nucleoplasm. Extending this theme to nucleostemin then raises the question of whether nucleostemin exerts its functions in the nucleolus proper or whether its asso-

ciation with the nucleolus is primarily a means to regulate its concentration in the nucleoplasm. Either scenario is plausible and it will be important to resolve this issue, especially since there is at present no evidence to think that nucleostemin is involved in ribosome biogenesis or assembly.

The demonstration of a GTP-driven retention cycle is a clear precedent for how nucleolar localization can be tightly regulated and potentially linked to signaling pathways. But how general is control of nucleolar localization by GTP cycles? A search of the Lamond database indicates that ~3% of the over 700 known nucleolar proteins contain a GTP-binding or closely related motif. This estimate suggests that direct control by GTP cycles is a relevant but not a major pathway for nucleolar localization. On the other hand, as pointed out by Tsai and McKay, GTP-controlled localization of some nucleolar proteins might indirectly affect the distribution of other proteins. The B23 protein, for example, does not contain any obvious GTP binding motives, but its dynamic retention is affected by alterations in cellular GTP levels, suggesting that nucleolar proteins can mutually affect their localization.

The involvement of a GTP-driven cycle in determining nucleolar residency is important as it is the first true molecular mechanism for nucleolar localization. The finding is conceptually important because of its demonstration that retention, rather than active targeting, determines localization. Furthermore, the results will be important in understanding the cellular role of nucleostemin in proliferation. But maybe most importantly, the involvement of a GTP cycle will hopefully be an inspiration to the field to finally begin to systematically address the long overdue question of how cellular signal transduction pathways and localization to subnuclear compartments are functionally linked.

I thank Y. Lam and A. Lamond for database search and communication of unpublished data; S. Gorski, P. Scaffidi, E. Meshorer, and M. Dundr for comments. T. Misteli is a fellow of the Keith R. Porter Endowment for Cell Biology.

Submitted: 6 December 2004

Accepted: 9 December 2004

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