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RESEARCH HIGHLIGHT

The Krüppel traffic report: Cooperative signals direct KLF8 nuclear transport

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The Krüppel-like transcription factor (KLF) family consists of 17 distinct family members involved in the regulation of diverse cellular processes including differentiation, cell proliferation, growth-related signal transduction, angiogenesis and apoptosis (recently reviewed in [1]). In addition to their currently known biologic roles, the discovery that at least one member of the family, KLF6, can be alternatively spliced into biologically active isoforms with antagonistic functions and a distinct subcellular localization pattern [2], highlights the fact that nuclear-cytoplasmic shuttling of KLF proteins may represent an additional layer of functional regulation and the possibility of an even more diverse and completely unexplored role for KLF family members in both health and disease.

The study by Mehta *et al.* [3] on KLF8 nuclear localization provides additional and novel findings on the signals and cooperativity between them which direct subcellular trafficking of KLF family members. Prior to discussing these findings it is worthwhile to review the basic modular structure that has been "classically" described for this family. Based on sequence comparison, the modular structure is shown to be retained even in evolutionarily distant homologues including those in *Zebrafish* [4], *Xenopus* [5] and *Drosophila* [6]. A central feature ascribed to all KLF family members, if alternative splicing is not considered, has been their possession of three characteristic domains. The first is a highly variable N-terminal activation domain. Post-translational modifications within this domain and interactions with other proteins through this domain are believed to underlie each KLF family member's ability to act as either an activator or repressor of transcription. Second, a C-terminal region containing three highly conserved C_2H_2 zinc fingers (ZFs) comprises the DNA binding domain. Finally, based originally on the presence of an enriched stretch of basic amino acids, a nuclear localization signal (NLS) region was predicted adjacent to the start of the zinc finger DNA-binding domain (Figure 1).

Functional NLSs were first demonstrated in KLF1 and KLF4. Surprisingly, while the NLS sequence was shown to be functional in each of these proteins [7, 8], the ZFs also were involved in determining subcellular localization. Specifically, all KLF1 ZFs were found to be necessary and sufficient to localize KLF1 in the nucleus as shown by either deletion [9] or different fusions of the ZFs to GFP [8]. For KLF4, all three ZFs together were also enough to localize GFP to the nucleus. In addition, deletion constructs revealed that combined ZF1 and the first part of ZF2 were also sufficient to localize GFP to the nucleus. When tested individually, each ZF could direct nuclear localization, however ZF3 alone was the weakest and its deletion from the full-length protein had no effect on nuclear localization [7].

More recent studies demonstrated that SUMOylation of KLF5 increases nuclear localization by inhibiting nuclear export signal (NES) activity. Mutations of the residues K151 and K202, which are located near an NES, inhibit SUMOylation, resulting in mislocalization of KLF5 to the cytoplasm. Moreover, this post-traslational modification also influences protein activity. The mislocalized mutants, unlike the wild type protein, lose the ability to promote anchorage-independent growth [10].

The authors from Mehta *et al.* [3] previously reported that KLF8 is also SUMOylated [11]. Different than KLF5, SUMOylation does not affect KLF8 nuclear localization but does regulate its function as a transcriptional repressor. To date, no other studies have addressed the regulation of KLF8 subcellular localization. Therefore, the study by Mehta *et al.* now provides some novel insights into the regulation of this transcription factor's nucleo-cytoplasmic transport.

Previous studies based on sequence homology have described two putative NLSs in KLF8 [12]. One of them is located immediately upstream of the

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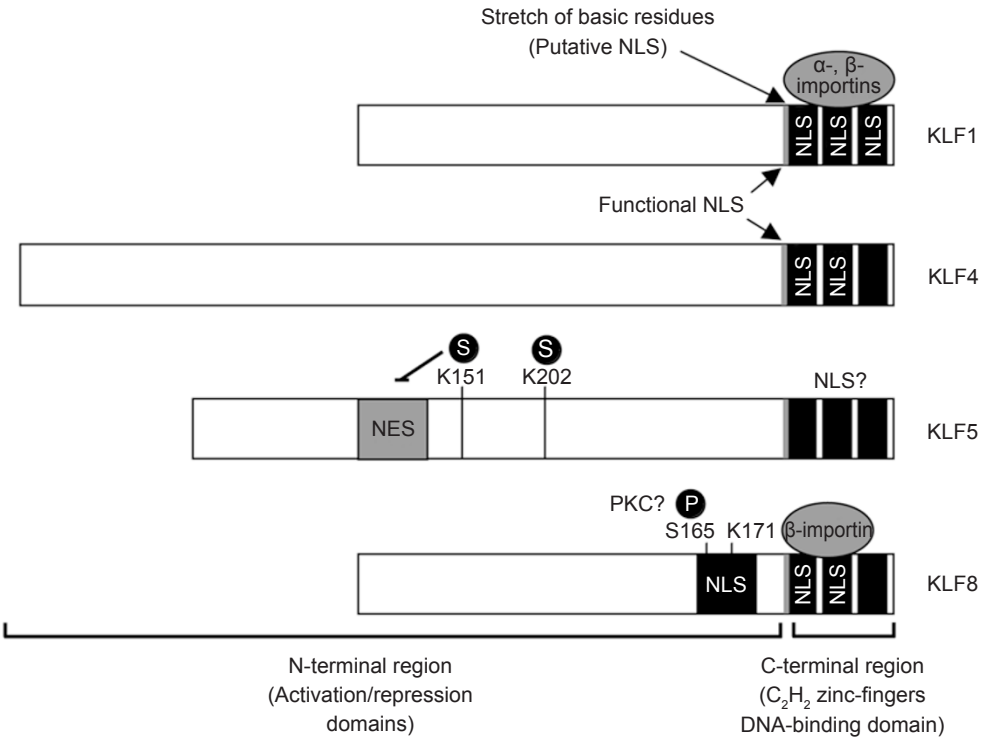

Figure 1 Modular structure of the KLF family members and domains controlling their subcellular localization. The cartoon shows the functional domains and post-traslational modifications related to nucleo-cytoplasmic transport identified in KLF1, KLF4, KLF5 and KLF8 [3, 7, 8, 9, 10]. NLS stands for Nuclear Localization Signal and NES, Nuclear Export Signal. The circles with an S indicate those residues that are SUMOylated, and the circle with a P, that the residue is phosphorylated. Binding to α- and β-importins is also shown.

ZFs (mNLS1), which corresponds to the stretch of basic amino acids present in other KLFs. The other, mNLS2, is located at the carboxy terminal of the KLF8 protein sequence. Mehta *et al.* demonstrated that these two sequences do not play a role in KLF8 nuclear transport as either deletion or mutation does not change KLF8 nuclear localization.

Instead, through a series of experiments Mehta *et al.* defined the presence of two functional and cooperative NLSs in KLF8 distinct from the originally presumed motifs [3]. The first, similar to what has been previously described for KLF1 and KLF4, was located within the ZF domain. Unlike the NLS in KLF1, in which all ZFs contribute to KLF1 nuclear localization, only the first two ZFs are required for nuclear transport. Deletion of either or both ZF1 and ZF2 in the full-length protein increases cytoplasmic localization. Similar to KLF4, targeted loss of ZF3 has no effect on subcellular distribution whereas similar to KLF1, KLF8 binds β-importin through interaction with its ZFs [8].

The second functional KLF8 NLS is novel and unique to this transcription factor. It is located within the N-terminal activation domain, between amino acids (aa) 151-200. The authors demonstrated that deletion of this region results in cytoplamic mislocalization. Moreover, this region contains two residues, S165 and K171, which seem to play a role in the regulation of KLF8 nuclear transport, as mutations of either increased KLF8 cytoplasmic localization. The S165 residue was suspected to be a PKC modification domain based on sequence similarity and indeed, treatment with a PKC inhibitor decreased nuclear localization. Future experiments will be necessary to elucidate whether PKC directly phosphorylates KLF8 and whether other post-traslational modifications may also control nucleo-cytoplasmic shuttling.

What functional consequences, if any, result from changes in KLF8 localization? As would be expected for a transcription factor, subcellular localization should be a critical determinant of function. KLF8 has been suggested to play important roles in human tumorigenesis through its ability to induce both cell cycle progression via activation of cyclin D1 [12] and in promoting epithelial to mesenchymal transition, oncogenic transformation and invasion [13, 14]. In part, this is regulated by transcriptional induction of KLF8

expression through activation of the focal adhesion kinase (FAK) pathway [12]. Therefore, dysregulation – for example, through mutation in tumor cells – may be an important, although as of yet unreported, pathogenic mechanism. In accord with this, Mehta *et al.* demonstrated that KLF8 requires both NLSs to up-regulate cyclin D1 expression and that mutations in S165 and K171 result in significant decreases in cyclin D1 promoter induction. These results correlate well with the results obtained by BrdU incorporation assays, which demonstrated decreased cellular proliferation compared to the wild type protein.

In summary, the study by Mehta *et al.* is the first to demonstrate that cooperation between both functional NLSs is necessary to regulate KLF8 nuclear transport and that nuclear localization is necessary for correct functioning as a transcription factor [3]. Therefore, despite the high degree of homology that all KLF proteins share within their ZF domains, it is becoming clear that their contribution to nuclear localization cannot simply be considered equal: the nuclear "traffic signals" encoded within these domains, while highly similar on a sequence level, are not functionally identical. Thus, beyond their current sequence-based [15], to truly appreciate the biology of this family, additional biochemical studies are needed to understand the rules of the road which ultimately regulate nucleo-cytoplasmic traffic of these transcription factors.

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