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Molecular cloning and functional characterization of mouse *Nxf* family gene products

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Abstract

Tap, a member of the evolutionarily conserved nuclear RNA export factor (NXF) family of proteins, has been implicated in the nuclear export of bulk poly(A)$^+$ RNAs. cDNAs encoding the mouse NXF proteins (Tap, NXF7, NXF2, and NXF3) were prepared, and the gene products were characterized in terms of their genomic organization, expression patterns, and biochemical properties. Mouse Tap was found to be ubiquitously expressed, whereas tissue- and developmental stage specific expression of mouse *Nxf2*, *Nxf3*, and *Nxf7* was observed. Although mouse Tap and NXF2 bound to the phenylalanine–glycine repeat sequences of nucleoporins, NXF7 and NXF3 did not. GFP-tagged mouse Tap and NXF2 were localized predominantly in the nucleus. In contrast, GFP-tagged NXF7 and NXF3 were localized exclusively in the cytoplasm. As shown for the human counterpart, disruption of the leucine-rich nuclear export signal or leptomycin B treatment abolishes the cytoplasmic localization of mouse NXF3. p15/NXT1, an essential cofactor for human Tap in the export of mRNAs, was able to bind to mouse Tap, NXF2, and NXF3, but NXF7 did not form a stable heterodimeric complex. Transient transfection experiments indicated that only mouse Tap and NXF2 enhance the nuclear export of an otherwise inefficiently exported mRNA substrate. The orthologous relationship between human and mouse *Nxf* genes is discussed on the basis of these data.

Keywords: Nucleocytoplasmic transport; Tap; Nuclear RNA export factor; mRNA; p15; Nucleoporin; X chromosome

Introduction

Since the cytoplasm and the nucleus are separate compartments in eukaryotic cells, mRNAs must be transported through the nuclear pore complex (NPC) into the cytoplasm before protein translation can occur. Conserved mRNA exporter proteins Tap (also referred to as nuclear RNA export factor 1, hereafter termed NXF1) and Mex67p, which interact with poly(A)$^+$ RNPs via the N and LRR domains and a series of nucleoporins containing phenylalanine–glycine (FG) repeats via the COOH domain, shuttle back and forth through NPC to transport bulk poly(A)$^+$ RNAs from the nucleus to the cytoplasm [1–6]. p15 (hereafter called NXT1) and its yeast counterpart Mtr2p, both of which are small proteins harboring the conserved NTF2-like fold [7,8], form very stable heterodimeric complexes with NXF1 and Mex67p, respectively. Heterodimer formation is required for these conserved mRNA export factors to transport mRNAs out from the nucleus [3,9–11]. It has been proposed that the binding of NXT1 and Mtr2p to the middle domain is required to maintain the correct folding of COOH-terminal halves of NXF1 and Mex67p to function as a binding platform to various FG-repeat sequences [8,11–14].

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It has been shown that, during or soon after transcription, precursor mRNAs (pre-mRNA) are associated with various proteins, in which a splicing factor UAP56 (or Sub2p in yeast) plays a critical role in converging transcription and subsequent downstream events, such as splicing and the nuclear export of mRNAs [15,16]. Initially UAP56 or Sub2p is recruited to pre-mRNAs via their association with the transcription elongation factor complex [17]. Subsequently, the release and reconstruction of the complex, which may occur simultaneously with splicing, promotes the association of various proteins on the mature and spliced mRNAs to form exon–junction complexes (EJCs) [18,19]. Aly (and its yeast counterpart Yra1p), which is the only known limiting factor for nuclear mRNA export involved in EJCs [20–22] and a subset of SR splicing factors [23], directly interacts with the mRNA exporters NXF1 and Mex67p, and, as a result, only fully processed mRNAs are exported to the cytoplasm. These physical and functional links among the transcription/splicing and the nuclear export factors are thought to have evolved as a safeguard against the nuclear export of inadequately processed mRNAs that may encode potentially toxic protein fragments [24].

Yeasts such as *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* harbor only one gene encoding the NXF family mRNA exporter (*MEX67* and *scMEX67*, respectively), whereas metazoans such as *Caenorhabditis elegans*, *Drosophila melanogaster*, and the human express more than two *Nxf* family gene products that share a similar domain organization with NXF1 in different tissues or in different phases of development [25–28]. The gene disruption of these conserved factors in *S. cerevisiae* [29] and the nematode [26] and the siRNA mediated knockdown of expression of individual *Nxf* genes in cultured fruit fly cells [27] indicate that *MEX67* and *NXF1* are essential genes, whereas other family members are dispensable either for cell viability or for the nuclear export of mRNAs. These data suggest that NXF1 is involved in the nuclear export of mRNAs that encode proteins with housekeeping functions, and that the other NXF family proteins are necessary for the nuclear export of tissue- and/or developmental stage specific mRNAs. However, a recent report indicated that *C. elegans* NXF2 is not involved in nuclear mRNA export, but rather it functions as a nuclear retention factor of a specific mRNA [30]. Another report indicated that human NXF1 plays roles not only in nuclear mRNA export but also in downstream events such as translation control of an unspliced viral mRNA [31]. Hence, some members of the NXF family of proteins, for which specific functions have not yet been assigned, may play more divergent functional roles.

The generation and characterization of "knockout" mice often provide an effective means for clarifying gene functions as well as the establishment of model systems for human diseases. For such experiments, it is essential to define the orthologue relationship between the human and mouse genes. On the other hand, a definition of the relationship between human and mouse *Nxf* gene family members has been hampered, partly because identification of the mouse *Nxf* gene products has not yet been completed [25,32]. In addition, as the sequence diversity of this protein family across different species is known to be extensive, it is somewhat risky to assume a relationship between NXF family members from different organisms based only on the available sequence data. To begin elucidating the specific functions of vertebrate *Nxf* family gene products by genetic approaches, we isolated a series of mouse *Nxf* gene products; characterized their genomic organization, expression patterns, and functional properties; and compared the data with those reported for the human *Nxf* gene products. Four different *Nxf* family genes were expressed in mice, and, as in the human counterparts, both mouse NXF1 and NXF2 behaved as bona fide mRNA exporter proteins. Indeed, we were able to show that they enhanced the nuclear export of an otherwise poorly exported model substrate. In contrast, mouse NXF3 and NXF7 were localized in the cytoplasm at a steady state and did not support the export of mRNA by themselves, possibly due to their inability to interact with adaptors and/or FG repeats, although NXF3 was found to shuttle between the nucleus and the cytoplasm. On the basis of these observations, the orthologous relationship between mouse and human *Nxf* family genes is discussed. In addition, our data suggest that cytoplasmically localized mouse NXF3 and NXF7 may have more divergent functional roles in mRNA metabolism.

Results

Molecular cloning of mouse Nxf gene products and their genomic organization

By searching the expressed sequence tags (ESTs) and the nucleotide sequence database, we found that the mouse expresses four different *Nxf* family genes. To begin characterizing the mouse *Nxf* family gene products, full-length cDNAs were obtained, as some of the sequences in the database were truncated or ambiguous and the full-length cDNA sequence for mouse NXF3 was missing (see Materials and Methods). By comparing the obtained cDNA and their corresponding genomic DNA sequences using the BLAST2 sequence alignment program, we established the genomic organization of the mouse *Nxf* genes. Mouse *Nxf1* was encoded on chromosome 16, whereas others form a gene cluster within an approximately 1.2-Mb region on the X chromosome, which is flanked by known marker genes such as α-galactosidase (*Gla*, GenBank Accession No. U34071) and myelin proteolipid protein (*Plp*, GenBank Accession No. NM_011123) (Fig. 1A). The organization of this region is well conserved between human and mouse genome, except that the human genome appears to harbor another *Nxf* family gene called *Nxf4* [25].