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Tool box: Plasmids for the expression or knockdown of human ARF Family GTPases (ARF/ARL/SAR) and their co-expression in bacteria with N-myristoyltransferases

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Abbreviations: ARF, ADP-ribosylation factor; ARL, ARF-like; MAP, methionine aminopeptidase; NMT, N-myristoyltransferase; ORF, open reading frame

This article is intended to inform researchers about a collection of ~200 plasmids recently made available through Addgene (www.addgene.com), that were generated to facilitate the study of human ARF family GTPases, including all 5 ARF and 2 SAR and an incomplete collection of ARF-like (ARL) proteins. They fall into 3 groups based upon usage; (1) ARF family GTPase expression in mammalian or bacterial cells, (2) N-myristoyltransferase co-expression in bacteria, and (3) pSUPER-based plasmids for siRNA knockdown of human ARF1, ARF3, ARF4, or ARF5. The majority of these plasmids direct the expression of human ARF family GTPases for study in mammalian cells or for purification from bacteria. The constructs are untagged or carry a few commonly used tags such as GFP, HA epitope, or V5-His6. These plasmids were engineered in the Gateway cloning system (Life Technologies) to allow ready insertion of other tags, thus the entry vectors are also provided. A group of 4 plasmids that direct expression of human N-myristoyltransferases, designed for co-expression in bacteria to allow N-myristoylation of recombinant proteins.[1,2] is included. We also provide a series of pSUPER-based plasmids.[3] proven useful in knockdown of ARF1-ARF5 in human cells.[4] A detailed summary of the construction of these plasmids and examples of their use is provided below and can be found in the cited references. Because ARF proteins in particular are very highly conserved (100% amino acid identity among several mammals including rodents), yet differ in DNA sequence, some of these plasmids may be useful in rescue experiments using gene deletion or knockdown.

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Arf Family GTPases in Gateway Vectors for Expression of Arf, Arl, And Sar Proteins in Mammalian and Bacterial Cells

With a long-term goal of studying the human ARF family and their functions in cells, we generated a collection of plasmids that direct expression of 21 different members of the human ARF family. The human ARF family today is known to include as many as 30 different members (Jeremy Wideman, Joel Dacks, and R. A. Kahn; manuscript in preparation). We obtained EST clones from public resources that included the entire open reading frames of ARF1, ARF3–6, ARL1–3, ARL4A/C/D, ARL5A/B, ARL6, ARL8A/B, ARL11, ARL14, ARFRP1, and SAR1A/B. These ORFs were amplified by PCR to add appropriate sites for recombination and insertion into the Gateway entry vector pDONR221. Two entry vectors were created for each ORF, one with and another without stop codons, to allow the generation of untagged or C-terminal tagged proteins. Each of these 42 entry vectors were sequence verified. We note in a few instances (ARL4D, ARL11, ARL14) single bp differences from the current NCBI entries, resulting in single missense mutations, but in each case our sequences were present in the EST clones from which they derived.

This set of 21 ARF family members were used to generate a total of 168 Gateway-derived plasmids, including the 2 sets of entry vectors (with and without stop codons), 4 sets for mammalian cell expression (untagged, or tagged at the C-terminus with HA, V5-His6, or GFP), and 2 sets for expression in bacteria (untagged or tagged at the C-terminus with V5-His6). The names and uses of these plasmids, along with gene names, aliases and NCBI Gene ID numbers are summarized in **Table 1**. Entry clones *with stop codons* were moved into pDEST47 or pDEST14 to generate plasmids for expression of untagged proteins in mammalian or bacterial cells, respectively. Entry clones *lacking a stop codon* were moved into (A) pDSHA, for expression of C-terminal HA tagged proteins in mammalian cells, (B) pDEST47, for expression of C-terminal GFP tagged proteins in mammalian cells, (C) pDEST40, for expression in mammalian cells of C-terminal V5-His6 tagged proteins, and (D) pET-DEST42, for expression in bacteria of C-terminal V5-His6 tagged proteins.

Table 1. Summary of the plasmids directing expression of human ARF family GTPases (ARF/ARL/SAR) in mammalian cells or bacteria, using the Gateway cloning system. The GTPase is listed, along with pseudonyms, NCBI Gene ID number, name of the plasmid as it appears in the Addgene collection, short description of the intended use, and reference in which it was first reported. Smaller collections of plasmids used for co-expression in bacteria of proteins of interest with N-myristoyltransferases 1 or 2 (NMT1/2) with or without methionine amino peptidase (MAP). The collection of pSUPER based plasmids directing expression of short hairpin RNAs (shRNAs) that deplete cells of human ARF1–5 are also included

GTPase	Pseudonyms	Gene ID	Addgene plasmid name	Description	Use
ARF1		375	pDONR221-ARF1	Entry vector: ARF1 w/ stop codon	Gateway entry vector
			pDONR221-ARF1-no stop	Entry vector: ARF1 w/o stop codon	Gateway entry vector
			pDSHA-ARF1-HA	ARF1-HA	Mammalian expression
			pDEST14-ARF1	ARF1	Bacterial expression
			pDEST47-ARF1-GFP	ARF1-GFP	Mammalian expression
			pET-DEST42-ARF1-V5-His6	ARF1-V5-His6	Bacterial expression
			pDEST40-ARF1-V5-His6	ARF1-V5-His6	Mammalian expression
			pDEST47-ARF1	ARF1	Mammalian expression
ARF3		377	pDONR221-ARF3	Entry vector: ARF3 w/ stop codon	Gateway entry vector
			pDONR221-ARF3-no stop	Entry vector: ARF3 w/o stop codon	Gateway entry vector
			pDSHA-ARF3-HA	ARF3-HA	Mammalian expression
			pDEST14-ARF3	ARF3	Bacterial expression
			pDEST47-ARF3-GFP	ARF3-GFP	Mammalian expression
			pET-DEST42-ARF3-V5-His6	ARF3-V5-His6	Bacterial expression
			pDEST40-ARF3-V5-His6	ARF3-V5-His6	Mammalian expression
			pDEST47-ARF3	ARF3	Mammalian expression
ARF4	ARF2	378	pDONR221-ARF4	Entry vector: ARF4 w/ stop codon	Gateway entry vector
			pDONR221-ARF4-no stop	Entry vector: ARF4 w/o stop codon	Gateway entry vector
			pDSHA-ARF4-HA	ARF4-HA	Mammalian expression
			pDEST14-ARF4	ARF4	Bacterial expression
			pDEST47-ARF4-GFP	ARF4-GFP	Mammalian expression
			pET-DEST42-ARF4-V5-His6	ARF4-V5-His6	Bacterial expression
			pDEST40-ARF4-V5-His6	ARF4-V5-His6	Mammalian expression
			pDEST47-ARF4	ARF4	Mammalian expression
ARF5		381	pDONR221-ARF5	Entry vector: ARF5 w/ stop codon	Gateway entry vector
			pDONR221-ARF5-no stop	Entry vector: ARF5 w/o stop codon	Gateway entry vector
			pDSHA-ARF5-HA	ARF5-HA	Mammalian expression
			pDEST14-ARF5	ARF5	Bacterial expression
			pDEST47-ARF3-GFP	ARF5-GFP	Mammalian expression
			pET-DEST42-ARF5-V5-His6	ARF5-V5-His6	Bacterial expression
			pDEST40-ARF5-V5-His6	ARF5-V5-His6	Mammalian expression
			pDEST47-ARF5	ARF5	Mammalian expression
ARF6		382	pDONR221-ARF6	Entry vector: ARF6 w/ stop codon	Gateway entry vector
			pDONR221-ARF6-no stop	Entry vector: ARF6 w/o stop codon	Gateway entry vector
			pDSHA-ARF6-HA	ARF6-HA	Mammalian expression
			pDEST14-ARF6	ARF6	Bacterial expression
			pDEST47-ARF4-GFP	ARF6-GFP	Mammalian expression
			pET-DEST42-ARF6-V5-His6	ARF6-V5-His6	Bacterial expression
			pDEST40-ARF6-V5-His6	ARF6-V5-His6	Mammalian expression
			pDEST47-ARF6	ARF6	Mammalian expression
ARL1	ARF1L	400	pDONR221-ARL1	Entry vector: ARL1 w/ stop codon	Gateway entry vector
			pDONR221-ARL1-no stop	Entry vector: ARL1 w/o stop codon	Gateway entry vector
			pDSHA-ARL1-HA	ARL1-HA	Mammalian expression
			pDEST14-ARL1	ARL1	Bacterial expression
			pDEST47-ARL1-GFP	ARL1-GFP	Mammalian expression
			pET-DEST42-ARL1-V5-His6	ARL1-V5-His6	Bacterial expression
			pDEST40-ARL1-V5-His6	ARL1-V5-His6	Mammalian expression
			pDEST47-ARL1	ARL1	Mammalian expression
ARL2	ARFL2	402	pDONR221-ARL2	Entry vector: ARL2 w/ stop codon	Gateway entry vector
			pDONR221-ARL2-no stop	Entry vector: ARL2 w/o stop codon	Gateway entry vector
			pDSHA-ARL2-HA	ARL2-HA	Mammalian expression
			pDEST14-ARL2	ARL2	Bacterial expression
			pDEST47-ARL2-GFP	ARL2-GFP	Mammalian expression
			pET-DEST42-ARL2-V5-His6	ARL2-V5-His6	Bacterial expression
			pDEST40-ARL2-V5-His6	ARL2-V5-His6	Mammalian expression
			pDEST47-ARL2	ARL2	Mammalian expression
			pDEST17-HA-ARL2	HA-ARL2	Mammalian expression

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Table 1. Summary of the plasmids directing expression of human ARF family GTPases (ARF/ARL/SAR) in mammalian cells or bacteria, using the Gateway cloning system. The GTPase is listed, along with pseudonyms, NCBI Gene ID number, name of the plasmid as it appears in the Addgene collection, short description of the intended use, and reference in which it was first reported. Smaller collections of plasmids used for co-expression in bacteria of proteins of interest with N-myristoyltransferases 1 or 2 (NMT1/2) with or without methionine amino peptidase (MAP). The collection of pSUPER based plasmids directing expression of short hairpin RNAs (shRNAs) that deplete cells of human ARF1–5 are also included *(Continued)*

GTPase	Pseudonyms	Gene ID	Addgene plasmid name	Description	Use
ARL3	ARFL3	403	pDONR221-ARL3	Entry vector: ARL3 w/ stop codon	Gateway entry vector
			pDONR221-ARL3-no stop	Entry vector: ARL3 w/o stop codon	Gateway entry vector
			pDSHA-ARL3-HA	ARL3-HA	Mammalian expression
			pDEST14-ARL3	ARL3	Bacterial expression
			pDEST47-ARL3-GFP	ARL3-GFP	Mammalian expression
			pET-DEST42-ARL3-V5-His6	ARL3-V5-His6	Bacterial expression
			pDEST40-ARL3-V5-His6	ARL3-V5-His6	Mammalian expression
			pDEST47-ARL3	ARL3	Mammalian expression
ARL4A	ARL4	10124	pDONR221-ARL4A	Entry vector: ARL4A w/ stop codon	Gateway entry vector
			pDONR221-ARL4A-no stop	Entry vector: ARL4A w/o stop codon	Gateway entry vector
			pDSHA-ARL4A-HA	ARL4A-HA	Mammalian expression
			pDEST14-ARL4A	ARL4A	Bacterial expression
			pDEST47-ARL4A-GFP	ARL4A-GFP	Mammalian expression
			pET-DEST42-ARL4A-V5-His6	ARL4A-V5-His6	Bacterial expression
			pDEST40-ARL4A-V5-His6	ARL4A-V5-His6	Mammalian expression
			pDEST47-ARL4A-GFP	ARL4A	Mammalian expression
ARL4C	ARL7, LAK	10123	pDONR221-ARL4C	Entry vector: ARL4C w/ stop codon	Gateway entry vector
			pDONR221-ARL4C-no stop	Entry vector: ARL4C w/o stop codon	Gateway entry vector
			pDSHA-ARL4C-HA	ARL4C-HA	Mammalian expression
			pDEST14-ARL4C	ARL4C	Bacterial expression
			pDEST47-ARL4C-GFP	ARL4C-GFP	Mammalian expression
			pET-DEST42-ARL4C-V5-His6	ARL4C-V5-His6	Bacterial expression
			pDEST40-ARL4C-V5-His6	ARL4C-V5-His6	Mammalian expression
			pDEST47-ARL4C	ARL4C	Mammalian expression
ARL4D	ARL9, ARL4L	379	pDONR221-ARL4D	Entry vector: ARL4D w/ stop codon	Gateway entry vector
			pDONR221-ARL4D-no stop	Entry vector: ARL4D w/o stop codon	Gateway entry vector
			pDSHA-ARL4D-HA	ARL4D-HA	Mammalian expression
			pDEST14-ARL4D	ARL4D	Bacterial expression
			pDEST47-ARL4D-GFP	ARL4D-GFP	Mammalian expression
			pET-DEST42-ARL4D-V5-His6	ARL4D-V5-His6	Bacterial expression
			pDEST40-ARL4D-V5-His6	ARL4D-V5-His6	Mammalian expression
			pDEST47-ARL4D	ARL4D	Mammalian expression
ARL5A	ARL5, ARFLP5	26225	pDONR221-ARL5A	Entry vector: ARL5A w/ stop codon	Gateway entry vector
			pDONR221-ARL5A-no stop	Entry vector: ARL5A w/o stop codon	Gateway entry vector
			pDSHA-ARL5A-HA	ARL5A-HA	Mammalian expression
			pDEST14-ARL5A	ARL5A	Bacterial expression
			pDEST47-ARL5A-GFP	ARL5A-GFP	Mammalian expression
			pET-DEST42-ARL5A-V5-His6	ARL5A-V5-His6	Bacterial expression
			pDEST40-ARL5A-V5-His6	ARL5A-V5-His6	Mammalian expression
			pDEST47-ARL5A	ARL5A	Mammalian expression
ARL5B	ARL8	221079	pDONR221-ARL5B	Entry vector: ARL5B w/ stop codon	Gateway entry vector
			pDONR221-ARL5B-no stop	Entry vector: ARL5B w/o stop codon	Gateway entry vector
			pDSHA-ARL5B-HA	ARL5B-HA	Mammalian expression
			pDEST14-ARL5B	ARL5B	Bacterial expression
			pDEST47-ARL5B-GFP	ARL5B-GFP	Mammalian expression
			pET-DEST42-ARL5B-V5-His6	ARL5B-V5-His6	Bacterial expression
			pDEST40-ARL5B-V5-His6	ARL5B-V5-His6	Mammalian expression
			pDEST47-ARL5B	ARL5B	Mammalian expression
ARL6	BBS3, RP55	84100	pDONR221-ARL6	Entry vector: ARL6 w/ stop codon	Gateway entry vector
			pDONR221-ARL6-no stop	Entry vector: ARL6 w/o stop codon	Gateway entry vector
			pDSHA-ARL6-HA	ARL6-HA	Mammalian expression
			pDEST14-ARL6	ARL6	Bacterial expression
			pDEST47-ARL6-GFP	ARL6-GFP	Mammalian expression
			pET-DEST42-ARL6-V5-His6	ARL6-V5-His6	Bacterial expression
			pDEST40-ARL6-V5-His6	ARL6-V5-His6	Mammalian expression
			pDEST47-ARL6	ARL6	Mammalian expression
ARL8A	GIE2, ARL10B	127829	pDONR221-ARL8A	Entry vector: ARL8A w/ stop codon	Gateway entry vector

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GTPase	Pseudonyms	Gene ID	Addgene plasmid name	Description	Use
			pDONR221-ARL8A-no stop	Entry vector: ARL8A w/o stop codon	Gateway entry vector
			pDSHA-ARL8A-HA	ARL8A-HA	Mammalian expression
			pDEST14-ARL8A	ARL8A	Bacterial expression
			pDEST47-ARL8A-GFP	ARL8A-GFP	Mammalian expression
			pET-DEST42-ARL8A-V5-His6	ARL8A-V5-His6	Bacterial expression
			pDEST40-ARL8A-V5-His6	ARL8A-V5-His6	Mammalian expression
			pDEST47-ARL8A	ARL8A	Mammalian expression
ARL8B	GIE1, ARL10C	55207	pDONR221-ARL8B	Entry vector: ARL8B w/ stop codon	Gateway entry vector
			pDONR221-ARL8B-no stop	Entry vector: ARL8B w/o stop codon	Gateway entry vector
			pDSHA-ARL8B-HA	ARL8B-HA	Mammalian expression
			pDEST14-ARL8B	ARL8B	Bacterial expression
			pDEST47-ARL8B-GFP	ARL8B-GFP	Mammalian expression
			pET-DEST42-ARL8B-V5-His6	ARL8B-V5-His6	Bacterial expression
			pDEST40-ARL8B-V5-His6	ARL8B-V5-His6	Mammalian expression
			pDEST47-ARL8B	ARL8B	Mammalian expression
ARL11	ARLTS1	115761	pDONR221-ARL11	Entry vector: ARL11 w/ stop codon	Gateway entry vector
			pDONR221-ARL11-no stop	Entry vector: ARL11 w/o stop codon	Gateway entry vector
			pDSHA-ARL11-HA	ARL11-HA	Mammalian expression
			pDEST14-ARL11	ARL11	Bacterial expression
			pDEST47-ARL11-GFP	ARL11-GFP	Mammalian expression
			pET-DEST42-ARL11-V5-His6	ARL11-V5-His6	Bacterial expression
			pDEST40-ARL11-V5-His6	ARL11-V5-His6	Mammalian expression
			pDEST47-ARL11	ARL11	Mammalian expression
ARL14	ARF7, ARL10	80117	pDONR221-ARL14	Entry vector: ARL14 w/ stop codon	Gateway entry vector
			pDONR221-ARL14-no stop	Entry vector: ARL14 w/o stop codon	Gateway entry vector
			pDSHA-ARL14-HA	ARL14-HA	Mammalian expression
			pDEST14-ARL14	ARL14	Bacterial expression
			pDEST47-ARL14-GFP	ARL14-GFP	Mammalian expression
			pET-DEST42-ARL14-V5-His6	ARL14-V5-His6	Bacterial expression
			pDEST40-ARL14-V5-His6	ARL14-V5-His6	Mammalian expression
			pDEST47-ARL14	ARL14	Mammalian expression
ARFRP1	ARP, ARP1, ARL18	10139	pDONR221-ARFRP1	Entry vector: ARFRP1 w/ stop codon	Gateway entry vector
			pDONR221-ARFRP1-no stop	Entry vector: ARFRP1 w/o stop codon	Gateway entry vector
			pDSHA-ARFRP1-HA	ARFRP1-HA	Mammalian expression
			pDEST14-ARFRP1	ARFRP1	Bacterial expression
			pDEST47-ARFRP1-GFP	ARFRP1-GFP	Mammalian expression
			pET-DEST42-ARFRP1-V5-His6	ARFRP1-V5-His6	Bacterial expression
			pDEST40-ARFRP1-V5-His6	ARFRP1-V5-His6	Mammalian expression
			pDEST47-ARFRP1	ARFRP1	Mammalian expression
SAR1A	SAR1, Sara, SARA1, masra2	56681	pDONR221-SAR1A	Entry vector: SAR1A w/ stop codon	Gateway entry vector
			pDONR221-SAR1A-no stop	Entry vector: SAR1A w/o stop codon	Gateway entry vector
			pDSHA-SAR1A-HA	SAR1A-HA	Mammalian expression
			pDEST14-SAR1A	SAR1A	Bacterial expression
			pDEST47-SAR1A-GFP	SAR1A-GFP	Mammalian expression
			pET-DEST42-SAR1A-V5-His6	SAR1A-V5-His6	Bacterial expression
			pDEST40-SAR1A-V5-His6	SAR1A-V5-His6	Mammalian expression
			pDEST47-SAR1A	SAR1A	Mammalian expression
SAR1B	ANDD, CMRD, GTBPB, SARA2	51128	pDONR221-SAR1B	Entry vector: SAR1B w/ stop codon	Gateway entry vector
			pDONR221-SAR1B-no stop	Entry vector: SAR1B w/o stop codon	Gateway entry vector
			pDSHA-SAR1B-HA	SAR1B-HA	Mammalian expression
			pDEST14-SAR1B	SAR1B	Bacterial expression
			pDEST47-SAR1B-GFP	SAR1B-GFP	Mammalian expression
			pET-DEST42-SAR1B-V5-His6	SAR1B-V5-His6	Bacterial expression
			pDEST40-SAR1B-V5-His6	SAR1B-V5-His6	Mammalian expression
			pDEST47-SAR1B	SAR1B	Mammalian expression
NMT1			pMON-HsNMT1	NMT1	Bacterial co-expression
NMT2			pMON-HsNMT2	NMT2	Bacterial co-expression

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GTPase	Pseudonyms	Gene ID	Addgene plasmid name	Description	Use
NMT1 + Met AP			pMON-NMT1+MAP	NMT1 + MAP	Bacterial co-expression
NMT2 + MetAP			pMON-NMT2+MAP	NMT2 + MAP	Bacterial co-expression
HsARF1 shRNA			pSUPER-ARF1a	ARF1 shRNA	Knockdown in human cells
HsARF1 shRNA			pSUPER-ARF1b	ARF1 shRNA	Knockdown in human cells
HsARF3 shRNA			pSUPER-ARF3a	ARF3 shRNA	Knockdown in human cells
HsARF3 shRNA			pSUPER-ARF3b	ARF3 shRNA	Knockdown in human cells
HsARF4 shRNA			pSUPER-ARF4a	ARF4 shRNA	Knockdown in human cells
HsARF4 shRNA			pSUPER-ARF4b	ARF4 shRNA	Knockdown in human cells
HsARF5 shRNA			pSUPER-ARF5a	ARF5 shRNA	Knockdown in human cells
HsARF5 shRNA			pSUPER-ARF5b	ARF5 shRNA	Knockdown in human cells

We chose not to tag the N-terminus because at least some, perhaps all, ARF family GTPases use the N-terminus as a nucleotide and phospholipid sensitive switch.[5] that may be directly involved in binding to effectors (e.g., see Zhang, et al).[6] In addition, co- or post-translational modifications of the N-termini, including N-myristoylation of ARFs.[7–9] and ARL1.[10–13] and acetylation of ARL3 and ARL8s,[14–16] have been found to be essential for cellular functions. The one exception to the use of N-terminal fusions is HA-ARL2, as we have found this N-terminal extension inhibits mitochondrial import and facilitates resolution of cytosolic and mitochondrial effects of ARL2 (Laura Newman, Cara Schiavon, Richard A. Kahn; manuscript in preparation). Concerns over the use of C-terminal fusions of ARF family members have been reported,[17] and users of these constructs are advised to include whatever controls are possible to protect against artifacts resulting from protein over-expression and/or interference by the tag in protein-protein interactions and functions. Finally, members of the Kahn laboratory have used most of these plasmids over the past few years for a variety of purposes. Our data suggest that the HA tagged proteins express quite poorly and more variably so preference should be given to the GFP or V5-His6 versions before using the HA-tagged constructs. The problem may lie in the vector backbone as we have expressed HA-tagged ARF proteins from pCDNA3-based vectors without this problem.

Vectors for Co-Expression of N-Myristoyltransferase (NMT) With or Without Methionine Aminopeptidase (MAP) in Bacteria

N-myristoylation is the co-translational, covalent attachment of the saturated 14-carbon fatty acid myristate onto the N-terminal glycine of certain proteins, after cleavage of the initiating methionine.[18] Not all proteins with N-terminal glycines are N-myristoylated (e.g.,, ARL2 is not) yet many of those that are require the modification for function in cells.[5,7,9,19–21] ARFs use the N-myristate as a critical part of its nucleotide-dependent, and therefore reversible, membrane association mechanism. N-myristoylation of exogenously expressed proteins in mammalian cells is an efficient process; the proteins are completely acylated and the acyl group is thought to persist through the lifetime of the protein.[18,22,23] However, bacteria do not express NMTs and have relatively small pools of myristoyl coA (the other substrate of NMTs). Thus, to generate recombinant, N-myristoylated proteins in bacteria, it is necessary to co-express an NMT with the ARF/ARL protein of interest. Such a system was devised by Duronio, et al,[1] and allows dual selection of the NMT carrying plasmid with kanamycin and the selection of the NMT substrate (e.g., ARF1) with ampicillin. The use of different bacterial promoters also allows for independent induction of the NMT and the ARF/ARL substrate. Some NMT substrates can be purified from bacteria in a nearly completely acylated state by the use of this system. In contrast, we have found that human ARFs are incompletely (as low as a few %) N-myristoylated, resulting in a mixture of acylated and unmodified proteins that can be difficult to resolve. Among the approaches tried in our lab to increase the yield of the acylated species was the co-expression of methionine aminopeptidase (MAP) with the NMT, with the idea that more rapid or complete cleavage of the initiating methionine may result in higher stoichiometry of myristoylation. While we found this to be true, the effects were not as large as hoped.[2] Anyone using bacteria for expression of N-myristoylated proteins should be aware that incomplete acylation is common, though this is highly dependent on the substrate and the NMT used.

Plasmids were generated that direct expression of either human NMT1 or NMT2 and each construct was made with or without the ability to co-express the bacterial MAP, as described in detail in Van Valkenburgh, et al.[2] These 4 plasmids are listed in **Table 1**. Note that the plasmids in this collection are for expression/co-expression of human NMTs, while the original work from the Gordon lab used the yeast ortholog.[1,24] Some differences in specificity and efficiency of N-myristoylation in bacterial co-expression systems have been described.[2] While the Gordon lab has done an outstanding job of characterizing substrate specificities of NMTs for their substrates,[24–26] we recommend empirical testing of the best NMT. The value of co-expressing MAP should also be empirically determined, though we have observed no negative consequences due to MAP co-expression.

psuper-BASED PLASMIDS for SIRNA KNOCKDOWN of HUMAN ARF1, ARF3, ARF4, OR ARF5

Brummelkamp, et al.[3] developed the pSUPER vector for use in generating short interfering RNAs to knockdown expression of specific proteins in mammalian cells. This reference includes clear directions for the generation of plasmids that drive expression off the polymerase III H1-RNA promoter to generate 19nt of double stranded RNA with a hairpin that suppresses expression of genes of interest. We designed into the pSUPER vector 5 different targets directed toward human ARF1, ARF3, ARF4, or ARF5, and examined their effectiveness in depleting cells of specific ARFs. The two best for each ARF were then used in studies examining the consequences of single or dual knockdowns, as described in Volpicelli-Daley, et al.[4] The use of at least 2 sequence-independent targets was used to decrease chances of off-target effects being responsible for the observed phenotypes. The use of synthetic RNAs and more recently of the CRISPR/CAS9 technology appears to have superseded the use of plasmid-based siRNA, but the availability of verified plasmids for knockdown of human ARFs allows the generation of stably transfected cell lines in which the level of each ARF can be experimentally modulated. While the Kahn lab was the source for rabbit polyclonal antibodies specific to each of the human ARF proteins.[27] for many years that were useful in detecting and quantifying knockdowns, unfortunately these reagents are no longer available to the public as a result of depletion in stocks of rabbit sera.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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