Data Mining for Traffic Information

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Synopsis statement:

A commentary that highlights the use of large data sets to inform our view of membrane traffic. Using published proteomic data sets, we extract protein copy number information and localisation calls for RAB and SNARE family members. We examine which of these genes are essential as judged by CRISPR-based viability screens across multiple cell lines. Co-variance of CRISPR effects on viability across cell panels allows assignment of genes into functionally coherent clusters that can generate fresh insight.

Modern cell biology is now rich with data acquired at the whole genome and proteome level. We can add value to this data through integration and application of specialist knowledge. To illustrate, we will focus on the SNARE and RAB proteins; key regulators of intracellular fusion specificity and organelle identity. We examine published mass spectrometry data to gain an estimate of protein copy number and organelle distribution in HeLa cells for each family member. We also survey recent global CRISPR/Cas9 screens for essential genes from these families. We highlight instances of co-essentiality with other genes across a large panel of cell lines, that allows for the identification of functionally coherent clusters. Examples of such correlations include RAB10 with the SNARE protein Syntaxin4 (STX4) and RAB7/RAB21 with the WASH and the CCC (COMMD/CCDC22/CCDC93) complexes, both of which are linked to endosomal recycling pathways.

In this article we highlight a major development in modern cell biology; the incorporation of large scale datasets into our knowledge base. How can we leverage this information to inform our view of intracellular traffic? We would like to encourage more cell biologists to apply their expertise to help interpret this mass of new information. The community is now in possession of vaults of data, that few could have imagined at the launch of Traffic 20 years ago.

The **SNARE family** of proteins consists of 38 identified members in the human genome which provide a combinatorial code that ensures specificity of intracellular fusion. Commitment to a fusion event depends on the formation of a trans-SNARE complex that incorporates four cognate SNARE motifs contributed by both membranes (traditionally referred to as vesicle (v) and target (t) membranes). They are now classified as R-SNAREs (from arginine) and Q-SNAREs (from glutamine) according to the central residue within their SNARE motif. A functional SNARE complex is composed of one R-SNARE and 3Q-SNAREs, contributed by a representative from each of the Qa, Qb and Qc families (Table 1)[1,2]. Foundational studies systematically tested SNARE combinations for productive fusion events using an *in vitro* system, but the list of those which are validated and assigned to a particular pathway in mammalian cells is far from complete (Table 1)[3-7]. SNARE complex formation is regulated by the Sec1p/Munc18 (SM) family of proteins[8]. The **RAB family** of GTPases also contribute to compartmental identity through the orchestration of membrane trafficking, at least in part by the recruitment of tethering molecules that facilitate SNARE complex formation[9,10]. Proliferation of genomic data from a wide range of eukaryotes has enabled the phylogenetic reconstruction of both RAB and SNARE protein families[11-14].

SNARES and RABs: basic demographics

We and others have argued that appreciation of the underlying protein copy numbers is important to understanding cellular systems[15]. They provide essential parameters that inform computational models, but also provide a basis for common sense inferences. In the specific example of the ubiquitin system, which we have previously considered, the relative abundance of conjugating and deconjugating enzymes allows us to get a feel for the major regulators of ubiquitin homeostasis[16]. We refer the reader to the excellent textbook "Cell Biology by the numbers", which provides a series of fascinating vignettes designed to illustrate how numbers provide cell biologists with a "sixth sense"[17]. Recent mass spectrometry advances provide estimates of the number of each type of protein per cell. Here we filter published data for whole HeLa cells to build up an overview of SNARE and RAB protein populations[18]. Twenty nine SNAREs are expressed at more than 10,000 copies per cell. Of these the R-SNAREs are generally more abundant than their SNARE complex partners, culminating in SEC22B (32% of R-SNAREs >800,000 per cell) which is ~8-12 fold in excess of each cognate Q-SNARE (STX5, GOSR2, BET1) that together are implicated in ER-Golgi transport[6, 19]. Super-stoichiometric levels of SEC22B may reflect its proposed supplementary role as a negative regulator of the ER Qa SNARE, STX18[20]. RABs are generally more abundant than SNARE proteins. The highly abundant RAB1 isoforms (26% of all RABs, >3.7 x10^6 copies per cell) are also associated with ER to Golgi transport. Estimates from HeLa cells indicate that the ER represents about 4.4% of total cellular protein mass, similar to the plasma membrane (3.1%) and greater than endosomes (0.9%), Golgi/ERGIC (0.8%) and lysosomes (0.2%)[21]. Nine RABs (in decreasing order of abundance (RAB1B > RAB7A > RAB10 > RAB11B > RAB14 > RAB2A > RAB1A > RAB5C > RAB6A > RAB8A) are present at >500,000 copies per cell and collectively represent ~80% of total RABs.

Other proteomic efforts have looked at defining the contents of specific purified vesicle or organelle fractions. The contents of a single synaptic vesicle have been estimated to contain around 70 copies of the R-SNARE VAMP2[22]. The cognate SNAREs SEC22B-GOSR2-BET1-STX5 are the only SNARE proteins found to be enriched in the proteome of both *in vitro* generated COPI and COPII-coated vesicles associated with ER-Golgi transport[23]. Similarly the cognate set of VAMP4-VTI1A-STX6-STX16 has been identified in clathrin coated vesicle (CCV) fractions in addition to the Q-SNARE set of STX7-VT1B-STX8[24]. Quantitative western blotting of synaptic bouton fractions from rat brain shows roughly stoichiometric levels of RAB3 (18850 copies per bouton) and the major cognate SNAREs implicated in synaptic vesicle fusion (Table 1). Endosome linked SNAREs are found to be two orders of magnitude less abundant and also significantly below the levels of endosomal RABs, RAB5 (~630 copies per bouton) and RAB7 (~4500 copies per bouton)[25].

Another approach to assign proteins within a set of organelles is to cluster proteins according to their sedimentation/fractionation profiles across multiplexed experiments. Several

complementary studies using different cell types have recently been published, and show a high degree of correspondence[21, 26-28]. Each study has provided a web resource that allows easy query of a protein of interest (Table 2). As an example the study of Izthak et al. is able to confidently assign 5 SNAREs to endosomal compartments in HeLa cells (STX7, STX12, VTI1B, STX8, VAMP8)[21]. The Qa-SNAREs, which by many accounts are taken to define the target membrane for incoming vesicles, tend to be more readily assigned than the R-SNAREs. Amongst these, endosomal Qa-SNAREs (STX7+STX12 = 42%) and plasma membrane (STX2+STX3+STX4 = 21%) are over-represented relative to their compartmental protein mass, whilst ER (STX18, 10%) is highly under-represented. Might this be indicative of the relative membrane fusion activity at these organelles?

Proteins linked to SNAREs and RABs through systematic CRISPR/Cas9 screens

The development of whole genome CRISPR/Cas9 screening has generated systematic studies across more than 500 cell lines for cell viability, using standardised protocols and reagents (29-32). This has led to the identification of "core fitness genes" i.e. those that are essential for cell viability across the vast majority of cell lines. Figure 1 shows the data for SNARE proteins from the Qa family. STX5 and STX18 are clearly identified as essential genes whilst for the most part, loss of other family members is relatively benign. An exception is STX4, which is required for viability in a significant fraction of cells, thus displaying "context dependent essentiality". In this favourable condition one can then seek to identify genes that have correlated dependency profiles across a panel of cell lines. Kim et al. have conducted a statistical analysis across a large data-set of 342 cell lines (referred to as the Avana dataset[32]) to search for genes with such correlated essentiality scores[33]. They identified many clusters of genes with high functional coherence. Thus, for STX4, their analysis correlates its cell-dependency profile with the Qbc-SNARE SNAP23, SM family member Syntaxin binding protein 3 (STXBP3 otherwise known as MUNC18C) and RAB10. The identification of known STX4 interactors (SNAP23 and STXBP3) illustrates the coherence of cluster components. This example also provides a demonstration of the discovery and hypothesis building potential offered by this analysis, as STX4 and RAB10 have hitherto not been functionally linked. We argue for involvement in a common pathway essential to context dependent cell viability. Whilst RAB10 has been implicated in multiple trafficking pathways, we propose the uncovered linkage may reflect their shared influence upon endosome to plasma membrane trafficking[34,35]. One variation of this approach is to look for correlations between drug sensitivity and gene depletion phenotypes across cell panels instead of between two genes. STX4 together with the genetically linked proteins, SNAP23, STXBP3, GRHL2 (Grainyhead Like Transcription Factor 2) are found within the top five drug-gene associations for inhibitors of the ErbB2 family of receptor tyrosine kinases Erlotinib and Lapatinib[36]. In other

words cell lines dependent on STX4 for viability are especially sensitive to ErbB2 inhibitors. This provides compelling evidence that the receptor trafficking itinerary may dictate drug sensitivity.

The only other essential SNARE is the R-SNARE YKT6 that has been linked to the fusion of secretory vesicles with the plasma membrane[37]. Q_B SNAREs, BNIP1, GOSR2, BET1 and USE1, by virtue of their context dependent essentiality can be linked to other genes, but these throw little immediate light upon function. Other than STX4/SNAP23 discussed above there are no examples of cognate Q or R-SNAREs co-clustering, speaking to the redundancy built into the SNARE-dependent system.

There are more than 60 RAB family proteins in the human genome, yet we could find no example of a single core fitness gene. Recently a systematic survey of RAB protein knock-out in MDCK cells revealed that RAB1A/B and RAB5A/B/C paralogues are redundantly required to ensure cell survival and growth respectively[38]. In addition to RAB10 discussed above, several RABs display strong context dependence and some of these are found in co-essentiality clusters together with known regulators of their GTPase cycle or specific membrane fusion factors (Table 3). Note that these "genetic interactions" with RAB proteins, are so far not picked up as direct physical interactions reported in other useful databases, such as BIOGRID and STRING, which collate information on protein-protein interaction networks[39,40]. Loss of RAB18 in humans leads to a severe illness known as Warburg Micro Syndrome[41]. The RAB18 co-essentiality cluster clearly associates it with the three other genes linked to the same condition; RAB3GAP1 and RAB3GAP2 which form a complex, and a further GTPase activator TBC1D20.

RAB35 pairs with the small GTPase trafficking protein ARF6. Their relationship is understood and the co-ordination of their respective GTPase activation-inactivation cycles has been linked to phagocytosis events, endosomal recycling pathways and cytokinesis[42-45]. RAB5C is co-essential with two specific sub-units (VPS8 and TGFBRAP of the Class C core vacuole/endosome tethering (CORVET) complex, a known effector of RAB5 that is believed to mediate fusion between endosomes[46-47]. The only other component of this cluster is the SM protein VPS45. RAB6 is found to be co-essential with all four members (VPS51, VPS52, VPS53, VPS54) of the tethering factor Golgi-associated retrograde transport (GARP) complex, the GARP interactor protein, EIPR1, and the RAB6 specific GEF, RIC1-RGP1. The homologous complex was shown to be an effector of the Yeast RAB6 homologue Ypt6p and this interaction is conserved in human cells[48-50]. The best established role of this complex is to orchestrate fusion of endosome derived vesicles with late Golgi compartments[51].

RAB11A is best known as a key regulator of endosomal recycling to the plasma membrane[52]. However, with respect to co-essentiality, it segregates with Adaptor Related

Protein Complex 1 Subunit Gamma 1 (AP1G1), an adaptor protein constituent of Trans-Golgi Network (TGN) derived clathrin coated vesicles, destined for endosomes. Recent characterisation of RAB11A knockout cells reveals a defect in recycling of the cation-independent mannose 6-phosphate receptor (CI-M6PR) from late endosomes to the Golgi[53]. Thus we propose that the pre-eminent contribution of RAB11A to cell viability resides in the governance of bi-directional transport between the TGN and endocytic pathway.

Two other Rabs involved in endosome to plasma membrane recycling RAB7A and RAB21 are grouped in a highly coherent cluster together with elements of the Wiskott–Aldrich Syndrome protein and SCAR Homolog (WASH) complex, which orchestrates an actin-dependent recycling pathway (Figure 2)[54]. A recent study which used APEX2-RABs to identify associations through proximity based labelling supports these findings[55]. Both RAB7A and RAB21, but not other endosomal RABs (RAB4 and RAB5A), showed strong association with the WASH complex components. RAB7A is known to interact with the VPS35/29/26 retromer complex and mediate its recruitment to endosomes. Retromer then links directly to WASH complex through FAM21 (although this component of WASH is absent from the cluster). Also represented in the same cluster are three proteins belonging to the COMMD family (COMMD2, COMMD6, COMMD8). All ten COMMD protein family members are capable of interacting with coiled-coil domain–containing protein 22 (CCDC22) and CCDC93 to form a CCC (COMMD/CCDC22/CCDC93) complex[56-57]. CCDC22 links directly to the WASH complex by binding to FAM21, and WASH and CCC complexes co-operate with the recently identified Retriever Complex to recycle integrins and other membrane cargo proteins[58-59]. To our knowledge this is the first data that suggest a direct connection between RAB7A and RAB21 on this pathway.

We have highlighted insights that have emerged from large scale CRISPR/Cas9 based screens across many cancer cell lines, that assign a score to any given gene according to an effect on cell viability. To some extent this reflects that the driver for an effort on this scale has been the quest to discover new strategies for cancer treatment. Moving on, we expect similar screens that assess other cellular phenotypes such as cellular invasion, cell polarity and three dimensional organisation. This offers the prospect of discovering some of the same traffic regulators described above, that may now be clustered with a different set of proteins linked to that phenotype. The use of covariance across a panel of cell lines to make genetic based associations will likely be complemented by double knock-out based screens for synthetic lethality that are more analogous to the previous generation of genetic association screens, such as synthetic lethality in yeast. In this short article we have just scratched the surface of available data, but hope that the examples chosen illustrate the possibilities for a new era of integrative cell biology.

Figure 1: A. **corrected CRISPR score profile for Qa SNAREs across a panel of >500 cell lines (Avana2018q4)**. Each profile represents a corrected CRISPR score for each cell line arranged in rank order for each gene (32). The higher the score, the greater the linkage to cell viability. STX5 and STX18 can be considered essential genes, whilst STX4 shows context dependence, as it is only essential in a fraction of the tested cell lines. Profiles for all other family members have been included on the diagram but show very modest effects on cell viability. B. Co-essentiality network for STX4 available at https://hartlab.shinyapps.io/pickles/ and then selecting the Avana2017 q3 data set.

Figure 2: **RAB7A/RAB21 co-essentiality.** Clusters can be accessed at the following URL; https://hartlab.shinyapps.io/pickles/ and selecting the Avana2017 q3 data set

Table 1: **SNARE proteins, numbers, location and partners.**
Table indicating protein copy numbers per cell for SNARE proteins estimated by quantitative mass spectrometry in HeLa cells (18) or per synaptic bouton determined by quantitative western blotting of an isolated fraction (25). Colour coded cells indicate cognate SNARE complexes whose function has been validated in mammalian cell systems and for which the associated pathways (key) and references are also indicated. Final column indicates high confidence (bold) and lower confidence (italics) compartmental assignments based on multiplexed proteomics of HeLa cell fractions (21) (URL: http://mapofthecell.org). EE, early endosome, LE late endosome, MDV mitochondrial derived vesicle SV, synaptic vesicle, TGN, trans-Golgi Network, ER, endoplasmic reticulum, ERGIC, ER-Golgi intermediate compartment, PM, plasma membrane.

Table 2: **A list of useful websites providing access to relevant databases.**

Table 3: **Selected gene linkages to RAB proteins derived from co-essentiality networks:** All RAB proteins were screened for co-essentiality networks using Avana2017 q3 data together with the PICKLES interface (https://hartlab.shinyapps.io/pickles/) (33,60). Those RAB networks which incorporate SNAREs or other accessories to membrane fusion are highlighted along with regulators of the RAB protein itself (GAPs or GEFs).

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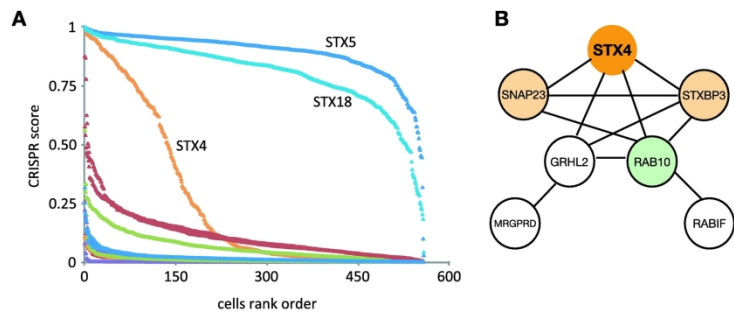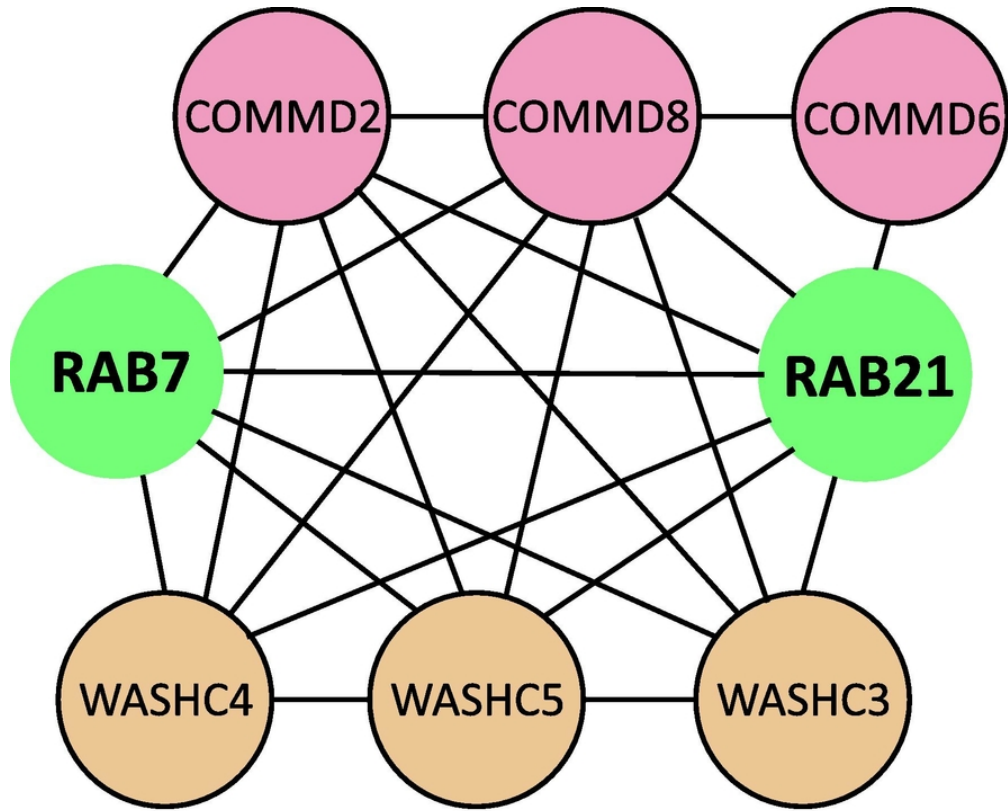
Figure 1

Figure 2

	protein copy numbers (HeLa)	protein copy numbers (synapse)	refs	map of the cell
STX1A		20096	61,62	
STX1B				
STX19				
TSNARE1				
STX12	109455	156		**endosome**
STX16	814	91	67,68	
STX17	44103		69,75	*Golgi*
STX18	41840		70,71	**ER**
STX2	13266			**PM**
STX3	22916		63,74	**PM**
STX4	54943		64,73	**PM**
STX5;STX5A	75579		6,19,65	**ERGIC**
STX7	73143		66,72	**endosome**
Qa-SNARES	**436058**			
BNIP1	115602		70,71	**ER**
GOSR1	43837		65	*Golgi*
GOSR2	65051		6,19	**ERGIC**
VTI1A	22957	51	67,68	
VTI1B	70614		66	**endosome**
Qb-SNAREs	**318062**			
STX6	94848	121	67	*endosome*
STX8	85729		66	**endosome**
STX10	70262		68	*ERGIC*
BET1	106159		6,19	**ERGIC**
BET1L	39801		65	
USE1	69829		70,71	**ER**
Qc-SNAREs	**466627**			
SNAP23	161700	266	63,64,74	**PM**
SNAP25	422	26686	61,62	
SNAP29	54955	77	69,72,73,75	
SNAP47	5330			
Qbc-SNAREs	**222407**			
VAMP1		3884		
VAMP2	10650	26448	61,62	*endosome*
VAMP3	623132		63,62,67,68,74	
VAMP4	31565	101	67	*endosome*
VAMP5	809			
VAMP7	176329		66,73,75	*endosome*
VAMP8	430771		66,69	**endosome**
YKT6	567512		65,72	*Golgi*
SEC22B	860690		6,19,70,71	*ER- high curvature*
R-SNAREs	**2701459**			

Key

Lysosome-PM	
Autophagy	
Golgi-ER	
LE-LE	
MDV-lysosome	
Autophagy	
endosome-TGN	
EE-EE	
endosome-PM	
EE-TGN	
ER-Golgi	
LE-lysosome	
Apical exocytosis	
SV-PM	
LE-Golgi	
endosome-PM	

website name	url	description
B10NUMBERs	https://bionumbers.hms.harvard.edu/aboutus.aspx	Curated database of numbers useful to cell biologists
Encyclopedia of protein dynamics	https://www.peptracker.com/accounts/login/epd/	Proteomics derived database from Lamond lab providing information on protein copy number estimates and turnover
NCI-60 proteome resource	http://129.187.44.58:7070/NCI60/	Comprehensive proteome analysis of the NCI-60 panel of cell lines
BioGRID	https://thebiogrid.org	protein-protein interaction repository
STRING	https://string-db.org	protein-protein interaction networks
Emililab	http://human.med.utoronto.ca	census of human soluble protein complexes
Bioplex	https://bioplex.hms.harvard.edu/index.php	Mass spectrometry derived database for protein-protein interactions
PICKLES	https://hartlab.shinyapps.io/pickles/	Pooled in vitro CRISPR knock-out library essentiality screens
DEPMAP	https://depmap.org/portal/ https://depmap.sanger.ac.uk	Cancer Dependency Map Project at the Broad and Sanger Institutes.
Map of the cell	http://www.mapofthecell.org	The HeLa cell spatial proteome
Prolocate	http://prolocate.cabm.rutgers.edu/index.cgi	Information on rat liver derived fractions
HyperLOPIT	https://proteome.shinyapps.io/hyperlopit-u2os2018/	The U2OS cell map
Human protein atlas	https://www.proteinatlas.org/humanproteome/cell	Antibody based characterisation of sub-cellular protein localisation

GENE CLUSTER	RAB protein	Tether	SM protein	SNARE protein	RAB GAP	RABGEF
20	RAB6A	VPS51 VPS52 VPS53 VPS54				RIC1-RGP1
61	RAB10		STXBP3	SNAP23, STX4		
98	RAB18				RAB3GAP1 RAB3GAP2 TBC1D20	
149	RAB5C	VPS8-TGFBRAP	VPS45			