Integration of Proteomics and Genomics in Platelets

A PROFILE OF PLATELET PROTEINS AND PLATELET-SPECIFIC GENES*[S]

J. P. McRedmond‡, S. D. Park‡, D. F. Reilly‡§, J. A. Coppinger‡, P. B. Maguire‡, D. C. Shields‡, and D. J. Fitzgerald‡¶

Platelets, while anucleate, contain RNA, some of which is translated into protein upon activation. Hypothesising that the platelet proteome is reflected in the transcriptome, we identified 82 proteins secreted from activated platelets and compared these, as well as published proteomic data, to the transcriptional profile. We also compared the transcriptome of platelets to other tissues to identify platelet-specific genes and used ontology to determine gene categories over-represented in platelets. RNA was isolated from highly pure platelet preparations for hybridization to Affymetrix oligonucleotide arrays. We identified 2,928 distinct messages as being present in platelets. The platelet transcriptome was compared with the proteome by relating both to UniGene clusters. Platelet proteomic data correlated well with the transcriptome, with 69% of secreted proteins detectable at the mRNA level, and similar concordance was obtained using two published datasets. While many of the most abundant mRNAs are for known platelet proteins, messages were detected for proteins not previously reported in platelets. Some of these may represent residual megakaryocyte messages; however, proteomic analysis confirmed the expression of many previously unreported genes in platelets. Transcripts for well-described platelet proteins are among the most platelet-specific messages. Ontological categories related to signal transduction, receptors, ion channels, and membranes are over-represented in platelets, while categories involved in protein synthesis are depleted. Despite the absence of gene transcription, the platelet proteome is mirrored in the transcriptome. Conversely, transcriptional analysis predicts the presence of novel proteins in the platelet. Transcriptional analysis is relevant to platelet biology, providing insights into platelet function and the mechanisms of platelet disorders. ***Molecular & Cellular Proteomics 3:133–144, 2004.***

Platlelets are anucleate cells formed from budding of megakaryocytes, from which they inherit much of their cytoplasmic contents. Messages for many platelet proteins are present in platelet RNA, including Fc receptors (1), plasminogen activator inhibitor-1 (2), protein kinase C isoforms (3), and chemokines (4). Indeed, coagulation factor XI is present in platelets as a platelet/megakaryocyte-specific splice variant (5). However, RNA in platelets was long regarded as a vestige of protein synthesis in the megakaryocyte (6).

Nevertheless, at least some platelet mRNA is functional: activated platelets synthesize proteins in a regulated manner, dependent on platelet activation and integrin engagement (7–10). These translated proteins include many of the more abundant proteins (11), but also the cytokine IL-1β (7) and the transcriptional regulator Bcl-3 (8). Rather than merely representing residual messages for megakaryocyte/platelet proteins, the profile of platelet mRNA may provide valuable insights into the biology of platelets and megakaryocytes, including information on the nonthrombotic functions of platelets.

We have interrogated gene arrays with platelet RNA and compared the results with a proteomic analysis of proteins secreted from platelets, as well as data from two previously published platelet proteomic studies (12, 13), to address whether the profile of platelet mRNA correlates with protein expression. Our findings confirm the general description of the platelet transcriptome provided by Gnatenko *et al.* (14). We also compare the platelet transcriptome with publicly available gene expression data from multiple tissues. We have identified individual messages that are relatively and absolutely platelet-specific as well as ontological categories over-represented in platelets.

MATERIALS AND METHODS

Platelet Preparation—Blood was drawn into one-sixth volume of buffer (38 mM citric acid, 75 mM sodium citrate, and 124 mM glucose), centrifuged at 180 $\times$ *g* for 10 min and the platelet-rich plasma (PRP)[1] carefully aspirated. PRP was centrifuged again at 180 $\times$ *g* in 50-ml tubes in a procedure optimized to remove remaining red and white cells. Platelet yields were 0.6–2.6 $\times$ 10^9 platelets from 50 ml of blood. Phase contrast microscopy showed these preparations to be devoid of the leukocytes and erythrocytes.

From the ‡Proteomics and Bioinformatics Cores, Department of Clinical Pharmacology, Royal College of Surgeons in Ireland, Dublin 2, Ireland

Received, July 2, 2003, and in revised form, October 6, 2003
Published, MCP Papers in Press, November 25, 2003, DOI 10.1074/mcp.M300063-MCP200

[1] The abbreviations used are: PRP, platelet-rich plasma; EST, expressed sequence tag; GP, glycoprotein; MudPIT, multidimensional protein identification technology; PF4, platelet factor 4; PBP, platelet basic protein; RANTES, regulated on activation normal T cell expressed and secreted; GO, Gene Ontology.

RNA Isolation and Hybridization—Purified platelets were centrifuged at 750 × *g* for 10 min and pellets resuspended in 1 ml of Tri-reagent (Sigma, Dublin, Ireland). RNA was isolated as per the manufacturer's instructions and resuspended in 25 μl of RNase-free water. Total RNA yields from 50 ml of blood draws were 1.1–4.5 (mean 2.5) μg. Reverse transcription PCR was used to analyze 125-ng aliquots of individual RNA samples. Presence of the low-abundance platelet tetraspannin CD151 was used as an index of platelet RNA, while the δ chain of the T cell antigen receptor-associated T3 complex was used as a marker of white cell contamination (Fig. 1*A*). Samples in which white cell contamination was detected were discarded. Platelet cDNA preparations were also negative for the pan-leukocyte tetraspanin CD53 (data not shown).

Pooled total RNA was prepared for hybridization to the HG-U95Av2 array (Affymetrix, Santa Clara, CA) as per the manufacturer's instructions. Briefly, 54 μg of total RNA from 23 donors was pooled and purified using an RNeasy column (Qiagen, Crawley, UK) to give 8 μg of purified total RNA. Double-stranded cDNA was synthesized and used to perform *in vitro* transcription, yielding 53 μg of labeled cRNA. This was fragmented and hybridized to the array according to the manufacturer's protocols. A second hybridization was performed using the same material.

Array Analysis—Array data was analyzed in the form of average difference values between perfect match and mismatch oligonucleotide sets, scaled so that the mean average difference value for the central 96% of values was equal to 200, as set out in the Gene Expression Atlas (expression.gnf.org/cgi-bin/index.cgi) (15).

Proteomic Analysis of Secreted Proteins—Washed platelets were prepared as previously described (16). Stirred platelets were stimulated with thrombin (0.5 U/ml) for 3 min and placed on ice. Platelets were removed by centrifuging twice at 1,000 × *g* for 10 min. The supernatant was cleared of microvesicles by ultracentrifugation for 1 h at 4 °C at 50,000 × g_{max} using a 50.4 Ti rotor (Beckman Instruments, Fullerton, CA).

The platelet supernatant (~300 μg protein) was precipitated overnight in acetone and centrifuged at 21,000 × *g* for 20 min. The pellet was resuspended in 8 M urea, 50 mM Tris-HCl, pH 8.5, at 37 °C and reduced with 1 mM dithiothreitol, followed by carboxyamidomethylation with 5 mM iodoacetamide for 1 h at 37 °C. The samples were diluted to 4 M urea with 50 mM NH_4HCO_3, pH 8.5, and digested with a 1:150 molar ratio of endoproteinase Lys-C at 37 °C overnight. The mixtures were further diluted to 2 M urea with 50 mM NH_4HCO_3, pH 8.5, supplemented with 1 mM $CaCl_2$, and incubated overnight with Poroszyme-immobilized trypsin beads at 30 °C, while rotating. The peptide mixtures were solid-phase extracted with SPEC-Plus PT C18 cartridges (Ansys Diagnostics; Lake Forest, CA) according to the manufacturer's instructions and stored at −80 °C until analyzed.

Multidimensional Protein Identification Technology (MudPIT)—A fully automated seven-cycle, 14-h MudPIT chromatographic procedure was established essentially as described (17, 18). Briefly, a high-performance liquid chromatography quaternary pump was interfaced with an LCQ DECA XP ion trap tandem mass spectrometer (ThermoFinnigan, San Jose, CA). A 150-μm i.d. fused silica capillary microcolumn (Polymicro Technologies, Phoenix, AZ) was pulled to a fine tip using a P-2000 laser puller (Sutter Instruments, Novato, CA) and packed with 10 cm of 5-μm Zorbax Eclipse XDB-C18 resin (Agilent Technologies, Mississauga, Canada) and then with 6 cm of 5-μm Partisphere strong cation exchange resin (Whatman, Clifton, NJ). Samples were loaded manually onto separate columns using a pressure vessel. Chromatography was carried out as described elsewhere (19). The SEQUEST algorithm (20) was used to identify proteins from tandem mass spectra. Ion state, XCorr, and DCn criteria yielding <1% false positive identification were used.

Linking Array Probesets Via UniGene Clusters—To allow comparisons between gene and protein expression data and to facilitate ontological analysis, probesets were linked to UniGene clusters. Accession numbers in the manufacturer's annotation were cross-referenced with UniGene (build 157, November 2002). This yielded links for 10,611 of the 12,625 Affymetrix probesets analyzed, while matches for a further 1,398 probesets were found by comparing gene names in the array with UniGene. The remaining 591 probesets were grouped where possible by cross-referencing accession numbers or descriptions. Because of multiple probesets for single genes on the Affymetrix chip, this process resulted in a total of 9,573 distinct probeset groups.

Linking Arrays to Proteomes—Proteomic data were linked to arrays via Swiss-Prot or GenBank accession numbers associated with proteins characterized from the proteome. These accession numbers were matched to UniGene clusters previously found to be associated with array probesets.

Platelet Specificity—To eliminate genes expressed highly in individual tissues, we screened curated expressed sequence tag (EST) library data for a variety of tissues, obtained from the TissueInfo website (21). Nonhuman, multiple-tissue, cancer, blood, and small (<500 ESTs) libraries were excluded. Libraries from the same tissue were combined and the ESTs linked to UniGene clusters, yielding 71 tissues with between 531 and 114,388 links to UniGene clusters. For each tissue, clusters accounting for >0.1% of the total number of ESTs (*i.e.* 1/1,000th of the transcriptome), and at least two ESTs were considered highly expressed. In this way, 1,983 of the 9,573 probeset groups on the array were filtered from the list of possible platelet-specific genes.

To identify genes expressed relatively specifically in platelets, we compared our gene expression data to 19 distinct noncancerous human tissues (excluding blood) in the Gene Expression Atlas. All expression datasets were normalized using a variance-stabilizing normalization (22) that converts signal intensities into *h* values, for which variance is approximately constant across the signal intensity range. For the normalization, 50% (the maximum possible value) of the genes were assumed to be differentially expressed across the set of hybridizations. Following transformation, for each of the probeset groups, *h* values were averaged across replicates. The platelet replicates were averaged to h_{plt}, and the nonplatelet, Gene Expression Atlas replicates for all tissues were averaged to give a single h_{gea} value. The difference in *h* values between platelet and nonplatelet tissue, $\Delta h = h_{plt} - h_{gea}$ was calculated for each gene. A high Δh indicates a gene expressed at a higher level in platelets than the other tissues in the Gene Expression Atlas.

Gene Ontology—Gene ontology organizes genes (on the basis of experimental evidence or homology) into categories according to subcellular localization, biological process, or biochemical function. By linking via UniGene, array probeset groups were assigned ontological terms defined by the Gene Ontology Consortium (www.geneontology.org). Linking to UniGene was achieved via two pathways. First, Swiss-Prot accessions with assigned Gene Ontology terms were obtained from the Gene Ontology database. The Swiss-Prot accessions were linked via associated cDNA sequence identifiers to UniGene. The second pathway linked Gene Ontology to UniGene via LocusLink from National Center for Biotechnology Information. Files loc2UG and loc2go downloaded from LocusLink (ftp.ncbi.nih.gov/refseq/LocusLink/) were used to perform the linkage.

Tests were performed to detect gene ontology terms for which the associated probesets were expressed at significantly higher or lower levels in platelets than in other human tissues. Intensities for each of the 9,573 probeset groups were averaged over all Gene Expression Atlas tissues, as before, and ranked. For every gene ontology term, the ranks in platelets and in the Gene Expression Atlas data for all associated probeset groups were compared using a nonparametric Wilcoxon signed rank test.

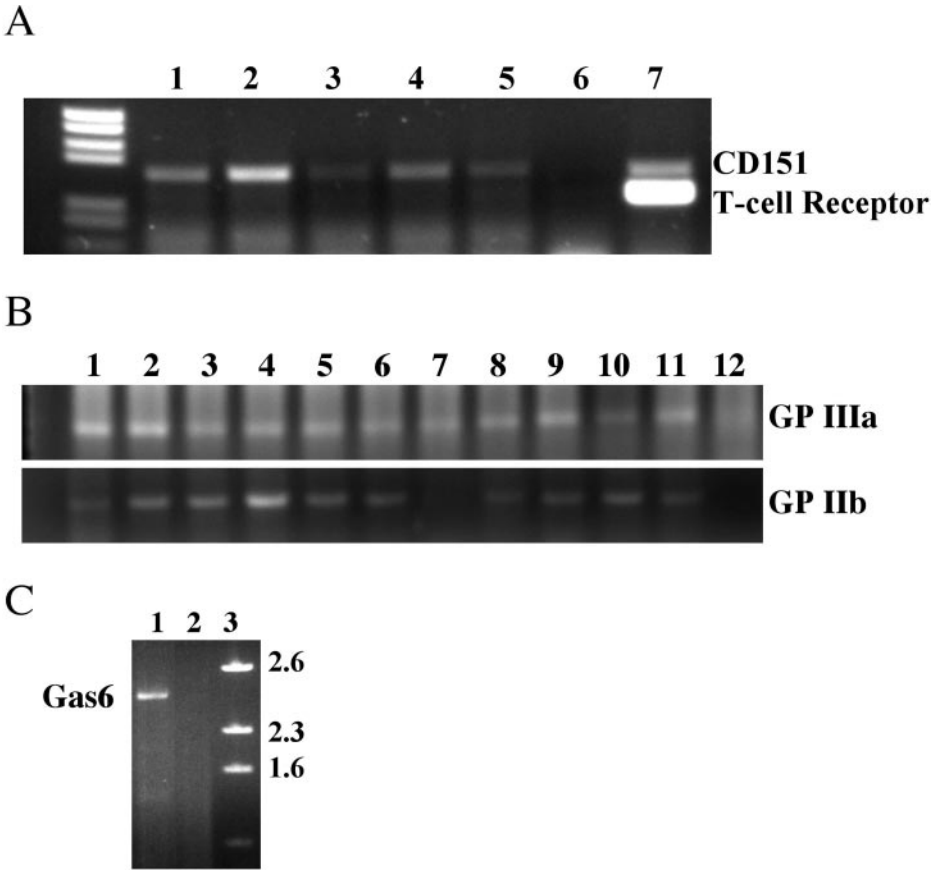

FIG. 1. **PCR analysis of platelet cDNA preparations.** *A*, PCR was performed to detect a platelet marker (CD151) and a nucleated cell maker (T cell receptor). *Lanes 1–5*, cDNA from individual platelet preparations. *Lane 6*, negative (no template) control. *Lane 7*, cDNA prepared from whole blood. Platelet RNA preparations are free of contaminating cell markers. *B*, PCR products from the 5′ end of GP IIb and GP IIIa were routinely detected in platelet cDNA synthesized by priming with oligo-dT at the 3′ end, demonstrating intact RNA up to 4 kb long. *Lanes 1–11*, individual platelet cDNA preparations. *Lane 12*, no template control. *C*, The entire coding region of Gas6, amplified from human platelet cDNA. *Lane 1*, platelet cDNA. *Lane 2*, no template. *Lane 3*, molecular weight markers, size indicated in kbp.

RESULTS

Platelet RNA—In preliminary investigations, we examined isolated platelet RNA for the presence of full-length messages. By using intron-spanning PCR primers directed against the 5′ end of cDNA species, in combination with oligo-dT-primed reverse transcription products, we detected full-length cDNAs (including 3′ untranslated regions) of several genes, including the platelet integrin components glycoprotein (GP) IIb (3.3 kb, forward primer GTCAGCTGGAGCGACGTCA, reverse primer CTGAATGCCCAAAATACGACG) and GP IIIa (4.0 kb, forward primer GCCGCTCTGGGCGACTGTGC, reverse primer CGCACTTGGATGGAGAAATTC), as well as the entire coding region of Gas-6 (2.5 kb (23), Fig. 1, *B* and *C*).

Array Analysis of Platelet RNA—The total signal intensity of a chip hybridized with platelet cRNA was lower than that seen with other tissues, perhaps due in part to the large proportion of mitochondrial RNA in platelets (Ref. 14 and unpublished data). This may have concentrated much of the label on a few probesets, resulting in a narrower distribution of probeset signal intensities.

Platelet hybridization data was normalized as described in "Materials and Methods" to allow comparison with other tissues, increasing the number of transcripts detected. Following normalization, we detected 3,978–4,022 positive probe sets out of 12,625 on the array (32% positive) in two samples, with excellent agreement between the hybridizations (Fig. 2). Mapping probeset sequences to UniGene revealed that this corresponded to 2,928 out of 9,573 (31%) unique transcripts. Subsequent analyses are all based on the 9,573 distinct probeset groups represented on the array. However, as transformation yields a similar number of "present" messages in all tissues, this may result in an overestimation of the diversity of the platelet transcriptome.

Using two other array systems, from MWG-Biotech (Ebersberg, Germany) and RZPD (Berlin, Germany), messages for 20–33% of the arrayed genes were detected in platelet RNA (data not shown). The incompatibility of diverse array formats makes comparisons between these systems difficult (24). The short oligonucleotides on Affymetrix arrays may lack sensitivity, particularly when hybridized to platelet RNA, which contains a large proportion of sequences from mitochondrial genes (14) lacking a complementary target on the array. The normalization described above may compensate for this.

Table I lists the 50 most abundant transcripts detected in platelets, indicating those for which a matching protein was found in platelets (see below). Of note, among the top 50 genes, along with messages for ubiquitous proteins (such as β_2-microglobulin (rank 3), β-actin (rank 10), ferritin (rank 11), and myosin light chain (rank 32)) are proteins known to be

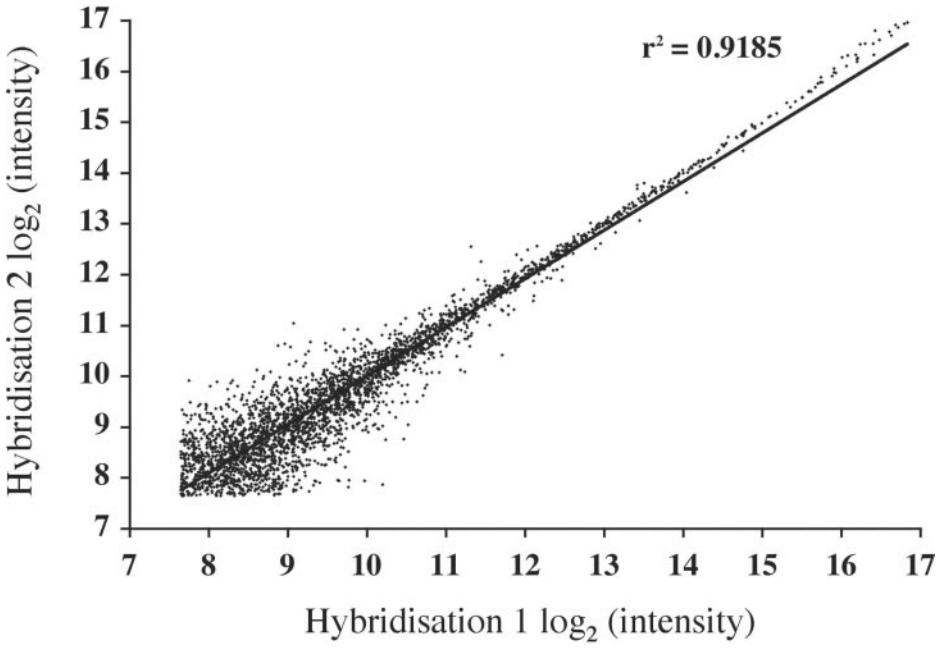

FIG. 2. **Correlation of log-transformed signal intensity from two Affymetrix microarray hybridizations using platelet RNA.** Plotted are those probesets with an average difference value on each of two hybridizations greater than 200, after transformation according to Gene Expression Atlas criteria (3,191 points).

highly expressed in platelets, such as platelet factor 4 (PF4, rank 8), platelet basic protein (PBP, rank 21), the von Willebrand factor receptor GP Ib (rank 24), the cytokine RANTES (rank 33), and GP IIb (rank 35), as well as numerous histones.

The 50 most abundant platelet transcripts in a recent publication by Gnatenko *et al.* (14) are in good agreement with ours. Indeed, nine of the top 10 and 45 of the top 50 genes are common to both lists (with a further three of the top 50 of Gnatenko *et al.* in the top 75 on our array). Thus array analysis of platelet RNA is highly reproducible in different laboratories.

Protein Expression of Platelet Messages—Initial analysis of proteins secreted from thrombin-activated platelets indicated that a small number of abundant proteins (including albumin and thrombospondin) dominate the platelet secreteome (48). Therefore, we used multidimensional protein identification technology (MudPIT) to allow a maximal separation of the platelet secreteome and a more comprehensive investigation of proteins present at a lower abundance. In samples from three separate donors, over 300 proteins were identified, with 82 observed in at least two of three samples. These 82 proteins formed our platelet "secreteome" dataset.

Linking the Affymetrix probesets and the proteins identified in the platelet proteome to UniGene facilitated the comparison of our array results with proteomic profiles. Of the 82 positively identified unique proteins in the platelet secreteome, 70 had matching probeset groups on the array. Of these 70 array-comparable proteins, messages corresponding to 48 (69%) were detected in platelet mRNA (Tables I and II). Strikingly, 17 out of the top 50 platelet messages were detected at the protein level in the platelet secreteome (Table I).

Similar agreement was found between the mRNA and protein profiles for two other proteomic analyses of platelets. O'Neill *et al.* (12) identified 536 protein features in the pI range 4–5, corresponding to 124 UniGene clusters. Of the 120 clusters represented on the array, 82 (68%) were present in platelets (Tables I and II). A further proteomic study by Marcus *et al.* (13) reported 186 tyrosine-phosphorylated platelet proteins that we mapped to 122 UniGene clusters, 64 of which were represented on the array. Of these, 44 (69%) were present in platelets at the message level (Tables I and II). The supplemental material gives a full list of transcripts for which a corresponding protein was found.

The proteomic and transcriptional datasets are strongly related. While the proteomic data used was not quantitative, transcripts corresponding to proteins detected in platelet samples have much higher transformed intensity (*h*) values than all messages on the array taken as a whole (Fig. 3, $p < 0.0001$ for each proteomic group *versus* all messages). Conversely, the presence of message in platelets can predict the existence of proteins. We have detected proteins by Western analysis of platelet lysates, predicted solely on the basis of array analysis of platelet RNA (data not shown).

One use of gene expression analysis is identifying as yet uncharacterized platelet messages and proteins. Platelets are known to contain numerous Rab proteins that are thought to be involved in regulated exocytosis of α and dense core granules (25). Several Rab GTPases including Rab3b, Rab4, Rab6, Rab8, and Rab27 have been demonstrated to be present in platelets (26), whereas mRNA for Rab31, Rab11a, and Rab32 have been identified recently (27). We found 15 members of the Rab family (including Rab proteins 4a, 4b, 6a, 11a, 27, 31, and 32) and four associated proteins among the 2,928 platelet-derived mRNA transcripts. Several of these messages, including that for Rab acceptor-1 protein, have not yet

TABLE I
Selected platelet messages

Platelet messages are listed in rank order of abundance. Average difference values were used to determine message signal intensity. Messages corresponding to proteins detected in platelet proteomic studies are indicated. Secreted proteins were identified in this study. Other proteins were matched to arrayed messages using accession numbers published by O'Neill *et al.* (12) and Marcus *et al.* (13).

Rank	Description	Secreted	O'Neill *et al.*	Marcus *et al.*	Average difference	Sample EST
1	Clusterin (complement lysis inhibitor)	x	x		122215	NM_001831
2	Ferritin H processed pseudogene				104363	J04755
3	β-2-microglobulin	x			96516	NM_004048
4	Neurogranin (PKC substrate, RC3)				88167	X99076
5	Thymosin, β 4	x			85945	NM_021109
6	Ornithine decarboxylase antizyme 1				84721	NM_004152
7	Transgelin 2	x		x	76181	NM_003564
8	PF4 (CXC chemokine 4)	x			75299	NM_002619
9	Hemoglobin, β	x			70247	NM_000518
10	Actin, β	x	x	x	58252	NM_001101
11	Ferritin, heavy polypeptide 1				57545	NM_002032
12	Glutathione peroxidase 1				56014	NM_000581
13	Osteonectin (SPARC)	x	x		54535	NM_003118
14	MHC, class I, C				49776	NM_002117
15	Talin 1	x	x		49456	NM_006289
16	Neuroendocrine secretory protein 55				47097	AJ009849
17	Coagulation factor XIII, A1	x	x		46360	NM_000129
18	H3 histone, family 3A				41091	NM_002107
19	α globin	x		x	35466	J00153
20	MutL homolog 3 (*E. coli*)				34880	NM_014381
21	PBP/CXC chemokine 7	x		x	33579	NM_002704
22	H2A histone family, member L				32426	U90551
23	14-3-3 β chain		x		30741	D78577
24	Platelet glycoprotein Ib β	x	x		30661	L20860
25	Nuclear receptor coactivator 4				30394	NM_005437
26	MHC, class I, E				28670	NM_005516
27	Cathepsin A		x		27850	AL008726
28	Progesterone receptor component		x		27840	NM_006667
29	Cofilin 1 (non-muscle)	x	x		27496	NM_005507
30	Myosin light chain 9, regulatory		x	x	27490	AK097235
31	Tubulin α		x		25316	X06956
32	Nonmuscle myosin light chain	x	x		25138	M22919
33	RANTES (CC chemokine 5)				24464	NM_002985
34	Ribosomal protein L41				24208	NM_021104
35	Integrin α 2b (platelet GP IIb)		x		24095	NM_000419
36	H2B histone family, member Q				23562	X57985
37	HERV RTVL-H neutral protease				23467	M27826
38	Ferritin light chain				21822	AL031670
39	Integrated membrane protein (MIC2)				20518	NM_002414
40	Filamin A, α	x	x	x	20126	NM_001456
41	Vinculin	x		x	19217	NM_014000
42	H2B histone family, member E				18214	Z83738
43	Prothymosin α				18115	NM_002823
44	H2A histone family, member A				17609	NM_021052
45	Dynein, cytoplasmic, light chain 1				17167	NM_003746
46	MHC, class I, A				16809	NM_002116
47	Weakly similar to histone H3.2				16661	AK057381
48	Actin-related 2/3 complex				16371	NM_005720
49	Ubiquitin C				16236	NM_021009
50	Monocyte-macrophage differentiation				15345	NM_012329

been reported in platelets. A Rab GDP dissociation inhibitor α (rank 250) and Rab 27b (rank 907) identified in the platelet proteome by Marcus *et al.* are in the platelet transcriptome (see supplemental material).

Platelet-specific Transcripts—Messages expressed exclusively in platelets, or at a higher level in platelets than in other tissues, might be expected to be particularly relevant to platelet physiology or specific targets for anti-platelet drugs.

TABLE II

Comparison of platelet proteomic and transcriptomic studies

Proteins identified by mass spectrometry were mapped to UniGene clusters as described in the text and compared to platelet gene expression data. Secreted proteins were identified in this study. Other platelet proteins were reported elsewhere: O'Neill *et al.* (12), Marcus *et al.* (13).

Study	Number of distinct proteins	No match on array	Number matching array	Present in transcriptome	Absent from transcriptome
		n (%)		*n* (%)	*n* (%)
Secreteome	82	13 (16%)	70	48 (69%)	22 (31%)
O'Neill *et al.*	124	4 (3%)	120	82 (68%)	38 (32%)
Marcus *et al.*	122	58 (48%)	64	44 (69%)	20 (31%)
Total			206	136 (66%)	70 (34%)

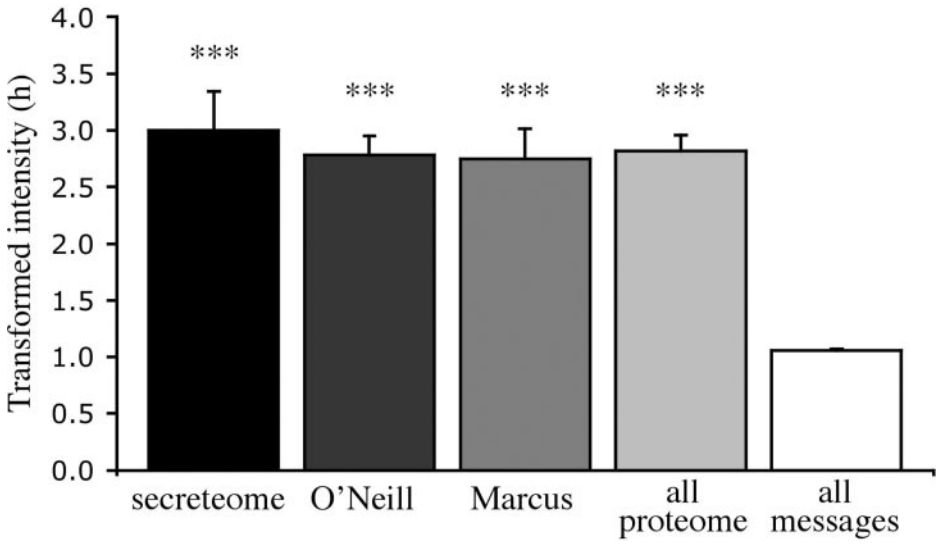

FIG. 3. **Platelet RNA is enriched with messages for platelet proteins.** Mean signal intensities (transformed average difference values) were calculated for array messages corresponding to proteins in the platelet proteome (the secreteome as described in this study, the proteomic studies of O'Neill et al. (12) and Marcus et al. (13), and all proteomic studies) and compared with the mean signal intensity for all messages on the array. Messages for platelet proteins are present at significantly higher levels in platelet RNA (*** indicates $p < 0.0001$ *versus* all messages).

A succession of methods were used to identify platelet-specific genes (Fig. 4). A total of 12,625 Affymetrix probesets were linked to 9,573 UniGene clusters. Of these, 1,983 were found to be highly expressed (more that 1/1,000th of transcriptome and at least two ESTs) in at least one of 71 non-blood normal tissue EST libraries. Of the remaining 7,590 transcripts, 2,110 were "present" in platelets according to Gene Expression Atlas criteria. Of these, 1,322 had a greater expression in platelets than in other tissues ($\Delta h > 0$). Ranking these in descending order of Δh gives a list of platelet-specific genes, the top 50 of which are shown in Table III.

Many of the most abundant platelet genes noted above are platelet-specific (PF4, 5th most platelet-specific; PBP, 7th; RANTES, 12th; GP IIb, 19th; GP Ib, 42nd). In addition, this platelet specificity analysis identifies the known platelet genes GP IX (21st), arachidonate 12-lipoxygenase (41st), PF4 variant 1 (34th), and the prostacyclin receptor (13th). Multiple histone genes are also designated "platelet-specific" by this analysis. The presence of histone messages in platelets is puzzling. Because platelets are anucleate, we would not expect them to contain any histone protein, and none was found in proteomic studies. Histones messages are rapidly degraded in other tissues (28), explaining their absence from other cell types in the Gene Expression Atlas. Their abundance in platelet RNA may be due to the peculiarities of the megakayocyte cell cycle. Mature megakaryocytes are polyploid (up to 128N), undergoing multiple G1-S phase transitions without mitosis. This stage of the cell cycle is associated with a burst of histone mRNA synthesis and prolonged histone message half-life (29). While they thus meet our bioinformatic criteria for platelet-specific messages, we are skeptical as to the biological significance of the elevated level of histone messages in platelets.

Ontology of Platelet Transcripts—We identified 1,529 extant nonsynonymous Gene Ontology (GO) terms corresponding to at least one UniGene cluster represented on the array. Of the 9,573 probeset groups, 7,778 were linked to at least one GO term; 876 terms were represented by at least 10 different probeset groups on the array. Wilcoxon signed rank tests were performed to identify the subset of these 876 GO terms for which the corresponding arrayed genes showed significantly different levels of expression between the Gene Expression Atlas tissues and platelets. A total of 112 GO terms showed significant overexpression and 109 significant underexpression ($p \leq 0.001$) in platelets relative to the Gene Expression Atlas tissues (Table IV).

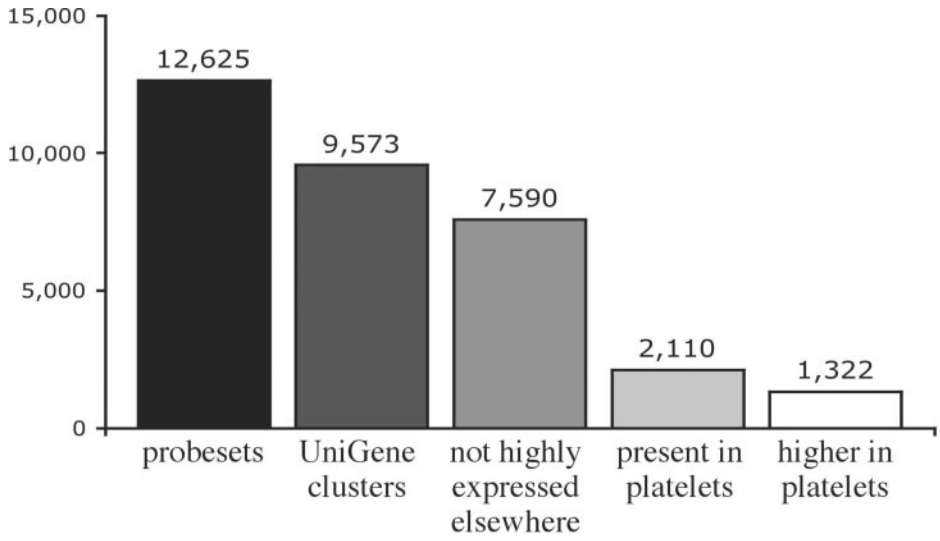

FIG. 4. **Identifying platelet-specific genes.** A total of 12,625 Affymetrix probesets were linked to 9,573 UniGene clusters. Of these, 1,983 were identified as being strongly expressed (>1/1,000th of the transcriptome) in at least one tissue EST library, as described in "Materials and Methods." Of the remaining 7,590 potentially platelet-specific genes, 2,110 were expressed in platelets, and 1,322 were expressed at a higher level in platelets than in the pool of tissues in the Gene Expression Atlas.

The number of comparisons made in ontological categories raises the risk of overestimating significant differences. However, Bonferroni correction for multiple comparisons would probably be too conservative in this case, particularly as not all ontology categories are independent (30). As a compromise, a significance level of $p \leq 0.001$ is used. When 876 ontology terms are compared between platelets and other tissues, one term might be expected to show significantly different gene expression by chance alone at the $p \leq 0.001$ level, whereas we observe 221 terms to be differentially expressed.

The gene ontology classification used here has a hierarchical structure. The single highest level term, "Gene Ontology" has three "second-level" daughter terms: "Biological Process," "Molecular Function," and "Cellular Component."

Daughter terms of the Biological Process category for which the associated genes are over-represented in platelets include "cell communication" and the corresponding daughter term "signal transduction." Also present at higher levels in platelets are genes associated with "stress response" and "response to external stimulus," their respective daughter terms "immune response" and "response to wounding," and genes associated with the term "hemostasis." Less predictably, genes associated with terms such as "development" are also over-represented. Notably under-represented in platelets are genes associated with the GO term "metabolism" and its daughter terms "protein biosynthesis," "mRNA processing," "regulation of translation," and "amino acid activation," all involved in protein synthesis (see Table IV).

Considering daughter terms in the Molecular Function category, "receptor binding," "receptor activity," "peptide binding," "cytokine activity," and "ion channel activity" (the terms are not mutually exclusive) tend to show higher expression in platelets than other tissues. Other tissues show a greater expression of genes in such categories as "ligase activity," "transcription factor binding," and "translation regulator activity" (see Table IV).

Considering Cellular Component GO terms, genes with an expression location within the cell (associated with the GO term "intracellular") as well as those in the ribosome tend to be under-represented, whereas those on the cell surface (GO term "plasma membrane") are highly expressed in platelets (see Table IV).

Taken as a whole, these ontology results show that platelets have a surfeit of receptor and signal transduction machinery, while they are lacking in messages for biosynthetic genes, particularly those involved in the various stages of protein metabolism.

DISCUSSION

The platelet transcriptome is complex, and analysis of platelet RNA tells us much about platelet function. Our results are in close agreement with recent data on the platelet transcriptome obtained using oligonucleotide arrays (14).

The correspondence between the platelet transcriptome and proteome validates the investigation of platelet RNA as a means to understanding platelet function. Fully 69% of secreted platelet proteins identified by us are detectable at the RNA level in platelets, demonstrating that platelet messages reflect the platelet protein profile. Similar results were obtained with two independent platelet proteomic analyses. This is a high level of agreement, given that proteomic analyses are less sensitive than those of mRNA (31).

Differences in the half-lives and transcription and translation rates of mRNA and protein mean that individual message and protein levels do not always correspond (31–33). Because gene transcription cannot occur in platelets, and translation is limited, we investigated how well the transcriptome reflected the proteome in circulating platelets. The datasets presented here differ from experimental analyses in other systems, because the platelet proteomic identifications are simply presence/absence and the array corresponds to a limited set of genes. In yeast and bacterial

TABLE III

The 50 most platelet-specific genes

UniGene clusters corresponding to Affymetrix probesets were compared to EST libraries to identify highly expressed genes. Transcripts making up over 1/1,000th of the transcriptome (and at least two transcripts) in at least one tissue EST library were eliminated from further analysis, as described in the methods (see Fig. 4). The remaining genes were listed in order of the difference between their expression level in platelets and a pool of 19 Gene Expression Atlas tissues. The 50 most platelet-specific genes are listed here. h_{plt}, transformed average difference value in platelets. h_{gea}, mean transformed average difference value in 19 Gene Expression Atlas tissues. Rank, position in list of platelet transcripts ranked by abundance.

	Description	h_{plt}	h_{gea}	Rank	Gene symbol	EST
1	H2A histone family, member A	5.64	−0.98	44	H2AFA	NM_021052
2	Neurogranin (PKC substrate, RC3)	7.24	0.87	4	NRGN	X99076
3	Pre-T-cell receptor α precursor	4.58	−1.77	157	PTCRA	NM_138296
4	HERV RTVL-H neutral protease	5.92	−0.19	37	HSRTVLH3	M27826
5	PF4 (CXC chemokine 4)	7.08	1.07	8	PF4	NM_002619
6	Hairy and enhancer of split homolog 2	4.45	−1.35	184	HES2	NM_019089
7	PBP/CXC chemokine 7	6.25	0.69	21	PPBP	NM_002704
8	Syntaxin 1A (brain)	5.24	−0.29	73	STX1A	NM_004603
9	Polyamine oxidase 1	4.52	−0.64	159	C20orf16	NM_019025
10	MutL homolog 3 (*E. coli*)	6.32	1.40	20	MLH3	NM_014381
11	Coagulation factor XIII, A1	6.60	1.72	17	F13A1	NM_000129
12	RANTES (CC chemokine 5)	5.43	0.77	33	CCL5	NM_002985
13	Prostacyclin receptor	3.49	−1.15	452	PTGIR	D38128
14	H2B histone family, member R	4.12	−0.40	143	H2BFR	X00088
15	ER to nucleus signaling 1	1.90	−2.58	2279	ERN1	NM_001433
16	Similar to mouse ADAM 3 (cyritestin)	2.85	−1.60	837		X89654
17	H2A histone family, member L	6.24	1.90	22	H2AFL	U90551
18	H4 histone family	3.55	−0.76	427	HSHIH4	X00038
19	Integrin α 2b (platelet GP IIb)	5.94	1.66	35	ITGA2B	NM_000419
20	Monocyte-macrophage differentiation	5.50	1.24	50	MMD	NM_012329
21	Glycoprotein IX (platelet)	5.12	0.92	83	GP9	NM_000174
22	cDNA clone IMAGE:649765 mRNA	4.96	0.88	99	HS1134159	AA216639
23	Mucin	3.31	−0.74	534		L07518
24	H3 histone family	4.22	0.17	98	H3FK	AL009179
25	HERV-H protein mRNA	3.89	−0.12	316	U92817	U92817
26	H2B histone family, member E	5.67	1.69	42	H2BFE	Z83738
27	SERCA3	3.51	−0.33	446	HSSERCA1	Y15724
28	GRB2-related adaptor protein 2	4.55	0.80	128	GRAP2	NM_004810
29	GPR12 G protein coupled-receptor	2.89	−0.83	789	GPR12	U18548
30	Transcription factor NF-E2	3.10	−0.58	630	NFE2	NM_006163
31	cDNA library, mRNA sequence	2.39	−1.28	1347	HSW26220	W26220
32	DD96 membrane associated protein	4.28	0.64	218	DD96	NM_005764
33	H2B histone family, member G	4.90	1.27	110	H2BFG	Z80779
34	Platelet factor 4 variant 1	3.80	0.20	346	PF4V1	M26167
35	Insulin-like growth factor binding protein	1.82	−1.78	2588	IGFALS	NM_004970
36	Phospholipase C, δ 1	2.08	−1.50	1936	PLCD1	NM_006225
37	Cathepsin A	6.09	2.55	27	PRTP	AL008726
38	H2B histone family, member Q	5.93	2.40	36	H2BFQ	X57985
39	H2B histone family, member H	5.08	1.57	87	H2BFH	Z80780
40	Occludin	4.19	0.71	236	OCLN	NM_002538
41	Arachidonate 12-lipoxygenase	4.53	1.08	169	ALOX12	NM_000697
42	Platelet glycoprotein Ib β	4.42	0.99	24	GP1BB	L20860
43	Carbonic anhydrase II	4.50	1.14	173	CA2	NM_000067
44	Cathelicidin antimicrobial peptide	1.73	−1.63	2225	CAMP	NM_004345
45	Similar to mouse WNT-6	5.23	1.91	74	HS458161	H12458
46	18S rRNA gene	3.34	0.03	388	HSRGE	M10098
47	Guanine nucleotide binding protein 11	5.09	1.78	84	GNG11	NM_004126
48	Chromosome 6 open reading frame 9	4.21	0.90	232	C6orf9	NM_022107
49	Membrane interacting protein of RGS16	2.22	−1.06	1583	HSU91321	U91321
50	Crystallin, γ C	1.67	−1.54	2702	CRYGC	NM_020989

studies, protein "quantification" is feasible and the organism's entire genome is on a single array (31, 34–38). In mammalian systems, more transcripts are invariably identified than proteins, due to the detection limits of proteomics technology. Comparing protein and message abundance, a correlation of 0.48 was found in a limited set of 19 human

TABLE IV

Ontology of gene expression in platelets and nonplatelet tissues

The table shows the gene expression categories with significant differences ($p \leq 0.001$) between platelets and tissues in the Gene Expression Atlas (GEA). The ranks of probeset groups belonging to every category were compared between platelets and the average of GEA tissues using Wilcoxon's ranked sign test, as described in "Materials and Methods." Categories with less than 25 probeset groups are not shown. Acc #, GO term accession number. *n*, number of probeset groups in GO term. GPCR, G-protein coupled receptor; HPO, haematopoietin; IFN, interferon; NA, nucleic acid; rec, receptor; sig. trans., signal transduction; act., activity; comp., complex.

Ranked higher in platelets			Ranked higher in GEA tissues		
Acc#	GO term	*n*	Acc#	GO term	*n*
9987	Cellular process	4068	8152	Metabolism	3958
7154	Cell communication	2172	5622	Intracellular	3820
16021	Integral to membrane	1813	5737	Cytoplasm	2192
7165	Signal transduction	1723	9058	Biosynthesis	649
4871	Signal transducer act.	1417	3723	RNA binding	380
5886	Plasma membrane	1270	16491	Oxidoreductase act.	368
7275	Development	1104	5739	Mitochondrion	342
5887	Integral to plasma membrane	958	6412	Protein biosynthesis	323
9605	Response to external stimulus	945	9059	Macromolecule biosynthesis	323
4872	Receptor activity	843	46907	Intracellular transport	311
7166	Cell surface rec. sig. trans.	690	5783	Endoplasmic reticulum	268
9607	Response to biotic stimulus	675	6886	Intracellular protein transport	246
6952	Defense response	617	5829	Cytosol	234
7242	Intracellular signaling cascade	612	8134	Transcription factor binding	233
4888	Transmembrane rec. act.	601	30529	Ribonucleoprotein comp.	197
6955	Immune response	561	5489	Electron transporter act.	183
7267	Cell-cell signaling	432	6091	Energy pathways	182
7186	GPCR signaling pathway	401	16070	RNA metabolism	182
9613	Response to pathogen	363	16874	Ligase act.	139
6811	Ion transport	362	6394	RNA processing	126
5102	Rec. binding	350	3754	Chaperone act.	116
9628	Response to abiotic stimulus	340	5840	Ribosome	107
6812	Cation transport	259	15077	Monovalent cation transporter	95
4930	GPCR act.	253	6397	mRNA processing	90
15267	Channel/pore class transporter	218	5740	Mitochondrial membrane	90
15268	α-type channel act.	215	15078	Hydrogen ion transporter act.	87
9611	Response to wounding	214	19941	Modified protein catabolism	82
9581	Perception of external stimulus	212	3729	mRNA binding	81
1584	Rhodopsin-like rec. act.	200	19866	Inner membrane	76
5216	Ion channel act.	198	45182	Translation regulator act.	73
30001	Metal ion transport	195	8135	Translation factor act., NA binding	72
19226	Transmission of nerve impulse	189	5830	Cytosolic ribosome	64
9582	Perception of abiotic stimulus	188	6445	Regulation of translation	49
7268	Synaptic transmission	187	15934	Large ribosomal subunit	41
15672	Monovalent cation transport	173	16651	NADH/NADPH oxidoreductase act.	40
5125	Cytokine act.	161	16282	Eukaryotic 43S preinitiation comp.	38
5261	Cation channel act.	135	15935	Small ribosomal subunit	31
42221	Response to chemical substance	134	16886	Phosphoric ester ligase act.	30
5244	Voltage-gated ion channel act.	96	502	Proteasome comp.	27
6935	Chemotaxis	87	16283	Eukaryotic 48S initiation comp.	27
42330	Taxis	87	6418	Amino acid activation	26
6968	Cellular defense response	82	8452	RNA ligase act.	26
8528	Peptide GPCR act.	80	16875	Carbon-oxygen bond ligase act.	26
1653	Peptide rec. act.	80	16876	Aminoacyl-tRNA ligase act.	26
7599	Hemostasis	72			
5267	Potassium channel act.	66			
7200	IP3 coupled G-protein signaling	59			
5126	HPO/IFN cytokine rec. binding	40			

liver proteins (39), while in cancer cells a poorer correlation was reported (40).

In the current study, protein levels were not quantified and therefore a direct comparison with other studies correlating protein and mRNA levels is not possible. However, when we compared whether a protein occurred in 0, 1, 2, or 3 of the proteomic studies (as an indicator of abundance) with rank order on the platelet mRNA array, the correlation was 0.22 among the 9,573 UniGene-linked proteins. Studies in yeast have revealed an increasing correlation between protein and message levels as gene expression increases (32). Similarly, in the current study when only proteins found in one or more proteome studies are considered, the correlation between occurrence in a proteome study with mRNA platelet rank is 0.36. This correlation is comparable with a correlation of 0.30 for mRNA and protein levels in a myeloid cell line, where Lian

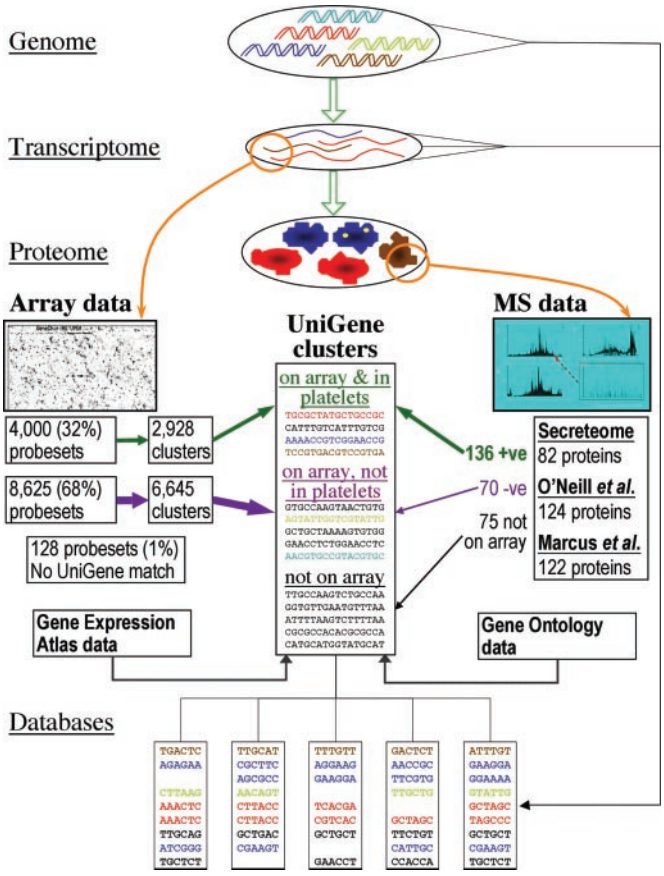

FIG. 5. **Information flow in experiments comparing transcriptome and proteome.** Transcriptional and proteomic data are compared by reference to a common database, namely UniGene. UniGene clusters information from multiple genomic and transcriptional sequence databases to give a single best sequence for each gene, which can be compared with diverse data formats, such as Affymetrix probesets, Swiss-Prot entries, and Gene Ontology databases. The transcriptome and proteome are sampled and their constituents identified using microarrays and liquid chromatography tandem mass spectrometry, respectively. Only messages for which probesets exist can be identified using arrays. Approximately one third of arrayed messages are present in platelets. All proteins identified by liquid chromatography tandem mass spectrometry are by definition (within technical limitations) present in the sample. Approximately half of proteins identified in three independent studies have a corresponding message in the platelet transcriptome, one-quarter are absent from the transcriptome, and one-quarter have no comparable sequence on the array.

et al. found 51 of 123 proteins (41%) to be present on an array of 12,488 probesets (33). While their bioinformatic analysis is somewhat different, the corresponding figures in the current study are 48 of 82 secreted proteins present (59%), for O'Neill *et al.* 82 of 124 proteins (66%), and for Marcus *et al.* 44 of 122 proteins (36%) (Table II).

The agreement we found between proteome and transcriptome in platelets is of interest, given the temporal displacement between the assumed protein synthesis (in the megakaryocyte) and RNA sampling (in circulating platelets). However, messages for ~30% of proteins were not detected, despite being represented on the array. RNA destruction following protein synthesis in the megakaryocyte, or in the circulating platelet, would result in proteins for which no message could be found. In addition, some platelet proteins (such as albumin and fibrinogen) are scavenged from plasma and might therefore not have any corresponding message in the platelet. However, the reasonable proportion of proteins detected at the message level by us and Lian *et al.*, as well as the persistence of histone messages, suggests that significant degradation of RNA does not occur in platelets.

Fig. 5 shows an overview of the relationship between transcriptional and proteomic data. In all, 136 out of 2,928 (4.6%) distinct platelet messages have been identified by proteomic analyses (Table II and Fig. 5). There was a small degree of overlap in the proteins identified in the three proteomic datasets, with 25 proteins being detected twice and six found in all three studies (see supplemental material). However, the three proteomic datasets are distinct subsets of the platelet proteome. We identified proteins secreted from activated platelets, O'Neill *et al.* confined their study to proteins in the pI range 4–5 (12), while Marcus *et al.* limited their analysis to phosphorylated proteins (13). Thus a major overlap between the proteome studies is not to be expected.

We would expect that as more comprehensive platelet proteomic studies are undertaken, the proportion of the platelet transcriptome shown to be represented at the protein level will increase. What fraction of the mRNAs is functional is unknown, but several proteins are synthesized in platelets and specific messages are associated with "translation-ready" polysomes in platelets (7), indicating that certain messages are maintained to allow their translation into functionally important proteins. It is not surprising given their 10-day lifespan that platelets are capable of generating proteins. To date, translation of mRNA for two main categories of proteins have been detected in platelets: first, proteins that are abundant, including major surface receptors such as GP Ib and GP IIb/IIIa and enzymes such as protein kinase C isoforms (3); and second, inflammatory proteins synthesized from RNA in activated platelets and released, such as Bcl-3 and IL-1β (7). These two messages are not abundant in platelets, leaving the biological significance of the commonest platelet messages open. While it is beyond the scope of this paper to determine their significance, we note that ferritin light chain has been reported in human atherosclerotic plaques, although no tissue source of mRNA was found (41). Ferritin light and heavy chains are among the most abundant platelet messages (Table I), raising that possibility that activated platelets adherent at sites of atherosclerosis could be a source of plaque marker proteins, through release of preformed protein or *de novo* synthesis. Components of the secreted platelet proteome, also present in the transcriptome, are also found histologically in atherosclerotic plaques (48).

We found that the most platelet-specific messages include

well-known platelet proteins, as well as chemokines, signaling molecules, and, surprisingly, histones. Ontological analysis bore out these initial findings. The categories of cell communication and signal transduction are more highly represented in platelets than a variety of other cell types. Multiple daughter terms of these large groupings are also highly expressed in platelets, including various categories of receptors and ion channels, reflecting the platelet's requirement to detect and respond to a wide variety of pro- and anti-coagulant signals, including a wide variety of soluble mediators, soluble and solid-phase adhesion molecules, and rheological and mechanical forces. The hemostasis category includes some classical coagulation factors (previously reported in platelets at the RNA level) as well as receptors involved in thrombosis, such as glycoproteins Ib, IX, and IIIa. The cytokine activty, defense, and immune response categories are also highly expressed in platelets. Three of the top 50 platelet messages (PF4, RANTES (4), and PBP, see Table I) are chemokines whose average rank in other tissues is much lower than that in platelets. The platelet has long been known to contribute to inflammatory responses through the release of mediators such as neutrophil-activating peptide-2 (42), and PF4 (43). Indeed, IL-1β from activated platelets has been implicated in endothelial cell inflammation (44–46). Platelets have also been reported to regulate the immediate inflammatory response in wound healing (47). While some of these proteins are released preformed, the demonstration of regulated IL-1β translation in platelets (7) suggests other platelet-derived cytokines could be produced this way also.

In conclusion, the considerable overlap between the platelet transcriptome and three separate proteomic studies confirms the validity of both datasets. Many of the proteins in the three proteomes have not been reported at the mRNA level in platelets, and some, such as the Rho GDP-dissociation inhibitor 2 (rank 97), WD repeat protein (rank 127), and adenylyl cyclase-associated protein 1 (rank 174) identified in the platelet secretome, have not previously been reported in platelets (see supplemental material). Some of these, such as hypothetical protein Q9BVV6 (rank 1744), are in the transcriptional dataset due to our analysis using Gene Expression Atlas, rather than standard Affymetrix criteria, and represent an extension of the platelet transcriptome beyond that previously reported. Thus, transcriptional analysis of platelets in comparison to other tissues reveals transcripts for proteins expressed during megakaryocyte function and platelet production (such as histones), as well as messages important in platelet function in thrombosis and inflammation. The transcriptome in platelets mirrors the profile of protein expression and therefore may provide novel insights when applied to the investigation of platelet disorders.

* This work was supported under the Programme for Research in Third Level Institutions, administered by the Higher Education Authority of Ireland, by the Enterprise Ireland Research Innovation Fund and the Health Research Board of Ireland. J. P. M. and P. M. are postdoctoral fellows of the Health Research Board and Enterprise Ireland, respectively. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[S] The on-line version of this article (available at http://www.mcponline.org/) contains supplemental material.

§ Current address: Center for Experimental Therapeutics, University of Pennsylvania, Philadelphia, PA 19104.

¶ To whom correspondence should be addressed: Department of Clinical Pharmacology, Royal College of Surgeons in Ireland, St. Stephens Green, Dublin 2, Ireland. Tel.: 011-353-1-4782165; Fax: 011-353-1-4022453; E-mail: dfitzgerald@rcsi.ie.

REFERENCES

1. Hasegawa, S., Pawankar, R., Suzuki, K., Nakahata, T., Furukawa, S., Okumura, K., and Ra, C. (1999) Functional expression of the high affinity receptor for IgE (FcϵRI) in human platelets and its intracellular expression in human megakaryocytes. *Blood* **93,** 2543–2551
2. Konkle, B. A., Schick, P. K., He, X., Liu, R. J., and Mazur, E. M. (1993) Plasminogen activator inhibitor-1 mRNA is expressed in platelets and megakaryocytes and the megakaryoblastic cell line CHRF-288. *Arterioscler. Thromb.* **13,** 669–674
3. Chang, J. D., Xu, Y., Raychowdhury, M. K., and Ware, J. A. (1993) Molecular cloning and expression of a cDNA encoding a novel isoenzyme of protein kinase C (nPKC). A new member of the nPKC family expressed in skeletal muscle, megakaryoblastic cells, and platelets. *J. Biol. Chem.* **268,** 14208–14214
4. Power, C. A., Clemetson, J. M., Clemetson, K. J., and Wells, T. N. (1995) Chemokine and chemokine receptor mRNA expression in human platelets. *Cytokine* **7,** 479–482
5. Hsu, T. C., Shore, S. K., Seshsmma, T., Bagasra, O., and Walsh, P. N. (1998) Molecular cloning of platelet factor XI, an alternative splicing product of the plasma factor XI gene. *J. Biol. Chem.* **273,** 13787–13793
6. Shaw, T., Chesterman, C. N., and Morgan, F. J. (1984) In vitro synthesis of low molecular weight proteins in human platelets: absence of labelled release products. *Thromb. Res.* **36,** 619–631
7. Lindemann, S., Tolley, N. D., Dixon, D. A., McIntyre, T. M., Prescott, S. M., Zimmerman, G. A., and Weyrich, A. S. (2001) Activated platelets mediate inflammatory signaling by regulated interleukin 1β synthesis. *J. Cell Biol.* **154,** 485–490
8. Weyrich, A. S., Dixon, D. A., Pabla, R., Elstad, M. R., McIntyre, T. M., Prescott, S. M., and Zimmerman, G. A. (1998) Signal-dependent translation of a regulatory protein, Bcl-3, in activated human platelets. *Proc. Natl. Acad. Sci. U. S. A.* **95,** 5556–5561
9. Pabla, R., Weyrich, A. S., Dixon, D. A., Bray, P. F., McIntyre, T. M., Prescott, S. M., and Zimmerman, G. A. (1999) Integrin-dependent control of translation: Engagement of integrin $\alpha_{IIb}\beta_3$ regulates synthesis of proteins in activated human platelets. *J. Cell Biol.* **144,** 175–184
10. Lindemann, S., Tolley, N. D., Eyre, J. R., Kraiss, L. W., Mahoney, T. M., and Weyrich, A. S. (2001) Integrins regulate the intracellular distribution of eukaryotic initiation factor 4E in platelets. A checkpoint for translational control. *J. Biol. Chem.* **276,** 33947–33951
11. Kieffer, N., Guichard, J., Farcet, J. P., Vainchenker, W., and Breton-Gorius, J. (1987) Biosynthesis of major platelet proteins in human blood platelets. *Eur. J. Biochem.* **164,** 189–195
12. O'Neill, E. E., Brock, C. J., von Kriegsheim, A. F., Pearce, A. C., Dwek, R. A., Watson, S. P., and Hebestreit, H. F. (2002) Towards complete analysis of the platelet proteome. *Proteomics* **2,** 288–305
13. Marcus, K., Immler, D., Sternberger, J., and Meyer, H. E. (2000) Identification of platelet proteins separated by two-dimensional gel electrophoresis and analyzed by matrix assisted laser desorption/ionization-time of flight-mass spectrometry and detection of tyrosine-phosphorylated proteins. *Electrophoresis* **21,** 2622–2636
14. Gnatenko, D. V., Dunn, J. J., McCorkle, S. R., Weissmann, D., Perrotta, P. L., and Bahou, W. F. (2003) Transcript profiling of human platelets using microarray and serial analysis of gene expression. *Blood* **101,** 2285–2293

15. Su, A. I., Cooke, M. P., Ching, K. A., Hakak, Y., Walker, J. R., Wiltshire, T., Orth, A. P., Vega, R. G., Sapinoso, L. M., Moqrich, A., Patapoutian, A., Hampton, G. M., Schultz, P. G., and Hogenesch, J. B. (2002) Large-scale analysis of the human and mouse transcriptomes. *Proc. Natl. Acad. Sci. U. S. A.* **99,** 4465–4470
16. Maguire, P. B., Wynne, K. J., Harney, D. F., O'Donoghue, N. M., Stephens, G., and Fitzgerald, D. J. (2002) Identification of the phosphotyrosine proteome from thrombin activated platelets. *Proteomics* **2,** 642–648
17. Wolters, D. A., Washburn, M. P., and Yates, J. R., 3rd. (2001) An automated multidimensional protein identification technology for shotgun proteomics. *Anal. Chem.* **73,** 5683–5690
18. Washburn, M. P., Wolters, D., and Yates, J. R., 3rd. (2001) Large-scale analysis of the yeast proteome by multidimensional protein identification technology. *Nat. Biotechnol.* **19,** 242–247
19. Peng, J., Elias, J. E., Thoreen, C. C., Licklider, L. J., and Gygi, S. P. (2003) Evaluation of multidimensional chromatography coupled with tandem mass spectrometry (LC/LC-MS/MS) for large-scale protein analysis: the yeast proteome. *J. Proteome Res.* **2,** 43–50
20. Eng, J. K., McCormack, A. L., and Yates, J. R., 3rd. (1994) An approach to correlate tandem mass spectral data of peptides with amino acid sequences in a protein database. *J. Am. Soc. Mass Spectrom.* **5,** 976–989
21. Skrabanek, L., and Campagne, F. (2001) TissueInfo: High-throughput identification of tissue expression profiles and specificity. *Nucleic Acids Res.* **29,** E102–102
22. Huber, W., Von Heydebreck, A., Sultmann, H., Poustka, A., and Vingron, M. (2002) Variance stabilization applied to microarray data calibration and to the quantification of differential expression. *Bioinformatics* **18,** (Suppl. 1), S96–S104
23. Angelillo-Scherrer, A., de Frutos, P., Aparicio, C., Melis, E., Savi, P., Lupu, F., Arnout, J., Dewerchin, M., Hoylaerts, M., Herbert, J., Collen, D., Dahlback, B., and Carmeliet, P. (2001) Deficiency or inhibition of Gas6 causes platelet dysfunction and protects mice against thrombosis. *Nat. Med.* **7,** 215–221
24. Kuo, W. P., Jenssen, T. K., Butte, A. J., Ohno-Machado, L., and Kohane, I. S. (2002) Analysis of matched mRNA measurements from two different microarray technologies. *Bioinformatics* **18,** 405–412
25. Wilson, S. M., Yip, R., Swing, D. A., O'Sullivan, T. N., Zhang, Y., Novak, E. K., Swank, R. T., Russell, L. B., Copeland, N. G., and Jenkins, N. A. (2000) A mutation in Rab27a causes the vesicle transport defects observed in ashen mice. *Proc. Natl. Acad. Sci. U. S. A.* **97,** 7933–7938
26. Karniguian, A., Zahraoui, A., and Tavitian, A. (1993) Identification of small GTP-binding rab proteins in human platelets: Thrombin-induced phosphorylation of rab3B, rab6, and rab8 proteins. *Proc. Natl. Acad. Sci. U. S. A.* **90,** 7647–7651
27. Bao, X., Faris, A. E., Jang, E. K., and Haslam, R. J. (2002) Molecular cloning, bacterial expression and properties of Rab31 and Rab32. *Eur. J. Biochem.* **269,** 259–271
28. Morris, T. D., Weber, L. A., Hickey, E., Stein, G. S., and Stein, J. L. (1991) Changes in the stability of a human H3 histone mRNA during the HeLa cell cycle. *Mol. Cell Biol.* **11,** 544–553
29. Harris, M. E., Bohni, R., Schneiderman, M. H., Ramamurthy, L., Schumperli, D., and Marzluff, W. F. (1991) Regulation of histone mRNA in the unperturbed cell cycle: Evidence suggesting control at two posttranscriptional steps. *Mol. Cell Biol.* **11,** 2416–2424
30. Perneger, T. V. (1998) What's wrong with Bonferroni adjustments. *Brit. Med. J.* **316,** 1236–1238
31. Greenbaum, D., Jansen, R., and Gerstein, M. (2002) Analysis of mRNA expression and protein abundance data: An approach for the comparison of the enrichment of features in the cellular population of proteins and transcripts. *Bioinformatics* **18,** 585–596
32. Gygi, S. P., Rochon, Y., Franza, B. R., and Aebersold, R. (1999) Correlation between protein and mRNA abundance in yeast. *Mol. Cell Biol.* **19,** 1720–1730
33. Lian, Z., Kluger, Y., Greenbaum, D. S., Tuck, D., Gerstein, M., Berliner, N., Weissman, S. M., and Newburger, P. E. (2002) Genomic and proteomic analysis of the myeloid differentiation program: Global analysis of gene expression during induced differentiation in the MPRO cell line. *Blood* **100,** 3209–3220
34. Yoshida, K., Kobayashi, K., Miwa, Y., Kang, C. M., Matsunaga, M., Yamaguchi, H., Tojo, S., Yamamoto, M., Nishi, R., Ogasawara, N., Nakayama, T., and Fujita, Y. (2001) Combined transcriptome and proteome analysis as a powerful approach to study genes under glucose repression in Bacillus subtilis. *Nucleic Acids Res.* **29,** 683–692
35. Eymann, C., Homuth, G., Scharf, C., and Hecker, M. (2002) Bacillus subtilis functional genomics: Global characterization of the stringent response by proteome and transcriptome analysis. *J. Bacteriol.* **184,** 2500–2520
36. Mader, U., Homuth, G., Scharf, C., Buttner, K., Bode, R., and Hecker, M. (2002) Transcriptome and proteome analysis of *Bacillus subtilis* gene expression modulated by amino acid availability. *J. Bacteriol.* **184,** 4288–4295
37. Wodicka, L., Dong, H., Mittmann, M., Ho, M. H., and Lockhart, D. J. (1997) Genome-wide expression monitoring in *Saccharomyces cerevisiae*. *Nat. Biotechnol.* **15,** 1359–1367
38. Greenbaum, D., Colangelo, C., Williams, K., and Gerstein, M. (2003) Comparing protein abundance and mRNA expression on a genomic scale. *Genome Biol.* **4,** 117.111–117.118
39. Anderson, L., and Seilhamer, J. (1997) A comparison of selected mRNA and protein abundances in human liver. *Electrophoresis* **18,** 533–537
40. Chen, G., Gharib, T. G., Huang, C. C., Taylor, J. M., Misek, D. E., Kardia, S. L., Giordano, T. J., Iannettoni, M. D., Orringer, M. B., Hanash, S. M., and Beer, D. G. (2002) Discordant protein and mRNA expression in lung adenocarcinomas. *Mol. Cell. Proteomics* **1,** 304–313
41. You, S. A., Archacki, S. R., Angheloiu, G., Moravec, C. S., Rao, S., Kinter, M., Topol, E. J., and Wang, Q. (2003) Proteomic approach to coronary atherosclerosis shows ferritin light chain as a significant marker: Evidence consistent with iron hypothesis in atherosclerosis. *Physiol. Genomics* **13,** 25–30
42. Piccardoni, P., Evangelista, V., Piccoli, A., de Gaetano, G., Walz, A., and Cerletti, C. (1996) Thrombin-activated human platelets release two NAP-2 variants that stimulate polymorphonuclear leukocytes. *Thromb. Haemost.* **76,** 780–785
43. Aziz, K. A., Cawley, J. C., and Zuzel, M. (1995) Platelets prime PMN via released PF4: Mechanism of priming and synergy with GM-CSF. *Br. J. Haematol.* **91,** 846–853
44. Bustos, M., Saadi, S., and Platt, J. L. (2001) Platelet-mediated activation of endothelial cells: Implications for the pathogenesis of transplant rejection. *Transplantation* **72,** 509–515
45. Kaplanski, G., Porat, R., Aiura, K., Erban, J. K., Gelfand, J. A., and Dinarello, C. A. (1993) Activated platelets induce endothelial secretion of interleukin-8 in vitro via an interleukin-1-mediated event. *Blood* **81,** 2492–2495
46. Hawrylowicz, C. M., Howells, G. L., and Feldmann, M. (1991) Platelet-derived interleukin 1 induces human endothelial adhesion molecule expression and cytokine production. *J. Exp. Med.* **174,** 785–790
47. Hilf, N., Singh-Jasuja, H., Schwarzmaier, P., Gouttefangeas, C., Rammensee, H. G., and Schild, H. (2002) Human platelets express heat shock protein receptors and regulate dendritic cell maturation. *Blood* **99,** 3676–3682
48. Coppinger, J. A., Cagney, G., Toomey, S., Kislinger, T., Belton, O., McRedmond, J. P., Cahill, D. J., Emili, A., Fitzgerald, D. J., and Maguire, P. B. (2004) Characterization of the proteins released from activated platelets leads to localization of novel platelet proteins in human atherosclerotic lesions. *Blood*, in press