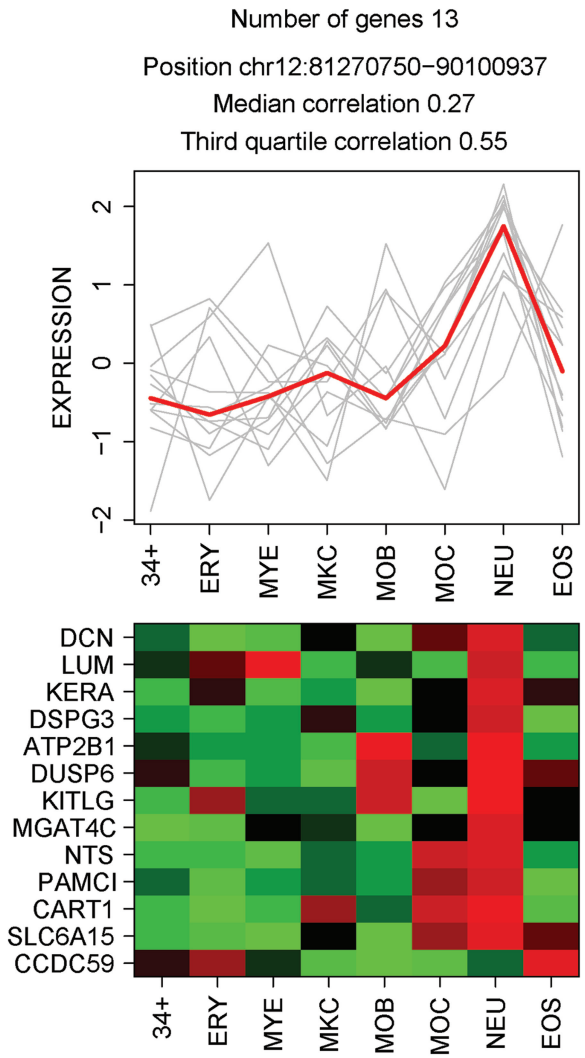

Figure 5. Example of a co-expressed region. Expression plot and heatmap, with genes ordered by genomic position, describing a region of chromosome 12 (81 270 750 – 90 100 937) harbouring 13 co-expressed genes. The red-bold line represents the median of expression profiles in the gene set. (34+: CD34+HSC; ERY: Erythroblasts; MYE: Myeloblasts; MKC: Megakaryoblasts; MOB: Monoblasts; MOC: Monocytes; NEU: Neutrophils; EOS: Eosinophils).

positions and sequence logo; (ii) alignment of patterns belonging to the motif; (iii) similarity with known TFBS and possible involvement in myelopoiesis; (iv) motif distribution along promoter sequences and uniformity test *P*-value; (v) number of sequences in which the motifs was found and total number of occurrences; and (vi) occurrence in subgroup of promoters belonging to highly co-expressed genes.

A total of 5325 significantly over-represented motifs were identified in 59 out of the 72 considered groups of gene promoters (Table 1).

One-third of discovered motifs are non-uniformly distributed along promoters. Since different evidences indicate non-uniform distribution of predicted motifs as a feature supporting their functional role, spatial distribution of over-represented motifs along promoters was also considered. Motifs with occurrences non-homogeneously distributed along promoter sequences were identified. Thus, each motif is associated to a bar graph representing the number of motif occurrences in six promoter regions and to a *P*-value from uniformity test. About one-third of discovered motifs (1772) resulted to be significantly non-uniformly distributed along the promoter sequence, with *P*-value $\leqslant 0.05$.

Presence of specific motifs in promoters may have a stronger effect on genes co-expression. As previously seen, each motif was identified because over-represented in a specific set of gene promoters and occurring in at least 30% of them. Expression patterns of genes whose promoters actually contain a specific motif were compared computing pairwise correlation coefficients. In this way, we were able to detect those motifs that occur in genes actually having correlations higher than the original gene set to which they belong (comparing the third quartiles of pairwise correlations), and that are supposed to be enriched in sequence elements truly functional in controlling gene expression. Thirty-five percent of discovered motifs (1881) fall in this category.

In addition, 11% of discovered motifs (578 in total) were found to have both properties of being non-uniformly distributed and occurring in highly correlated subgroups of genes. This, numerically manageable, fraction of discovered motifs may be envisaged as the group of best candidates for being functional regulatory elements.

Functional role in myeloid differentiation of discovered motifs is supported by previous knowledge. Motif-describing matrices were compared with those describing 142 known motifs (representing sequence elements binding known transcription factors, 29 of which correspond to 21 TF that are specifically involved in myeloid cell differentiation). Among the 5325 identified motifs, 19% (1009) are similar to at least one of the 142 known TFBS, retrieved from public databases and 187 of them are similar to binding sites for 21 selected TF relevant for myelopoiesis. On the other hand, 118 of the 142 known TFBS (83%), and 17 of the 21 binding sites of TF known to play a role in myeloid cells differentiation (81%) have a match with significantly over-represented motifs.

Comparison of motifs discovery results in CEG, CER, and CEMR gene promoters. In Supplementary Data file 6, the number of discovered motifs in CEG, CER and CEMR is shown as a bar plot. On average, there are 60.7 motifs in CEG, 134.8 in CER and 6.5 in CEMR sets. Apparently, more motifs are found in the promoters of genes co-expressed and co-localized (CER) than in groups of simply co-expressed genes (CEG). This observation also applies to subsets of motifs with biologically meaningful characteristics: i.e. those non-uniformly distributed, those occurring in highly correlated subgroups of genes, and those matching known TFBS. The number of motifs in the two considered CEMR is the lowest for all motif types.

In order to assess the significance of the difference between the number of over-represented motifs in CEG and CER, we considered a subset of CEG and CER with

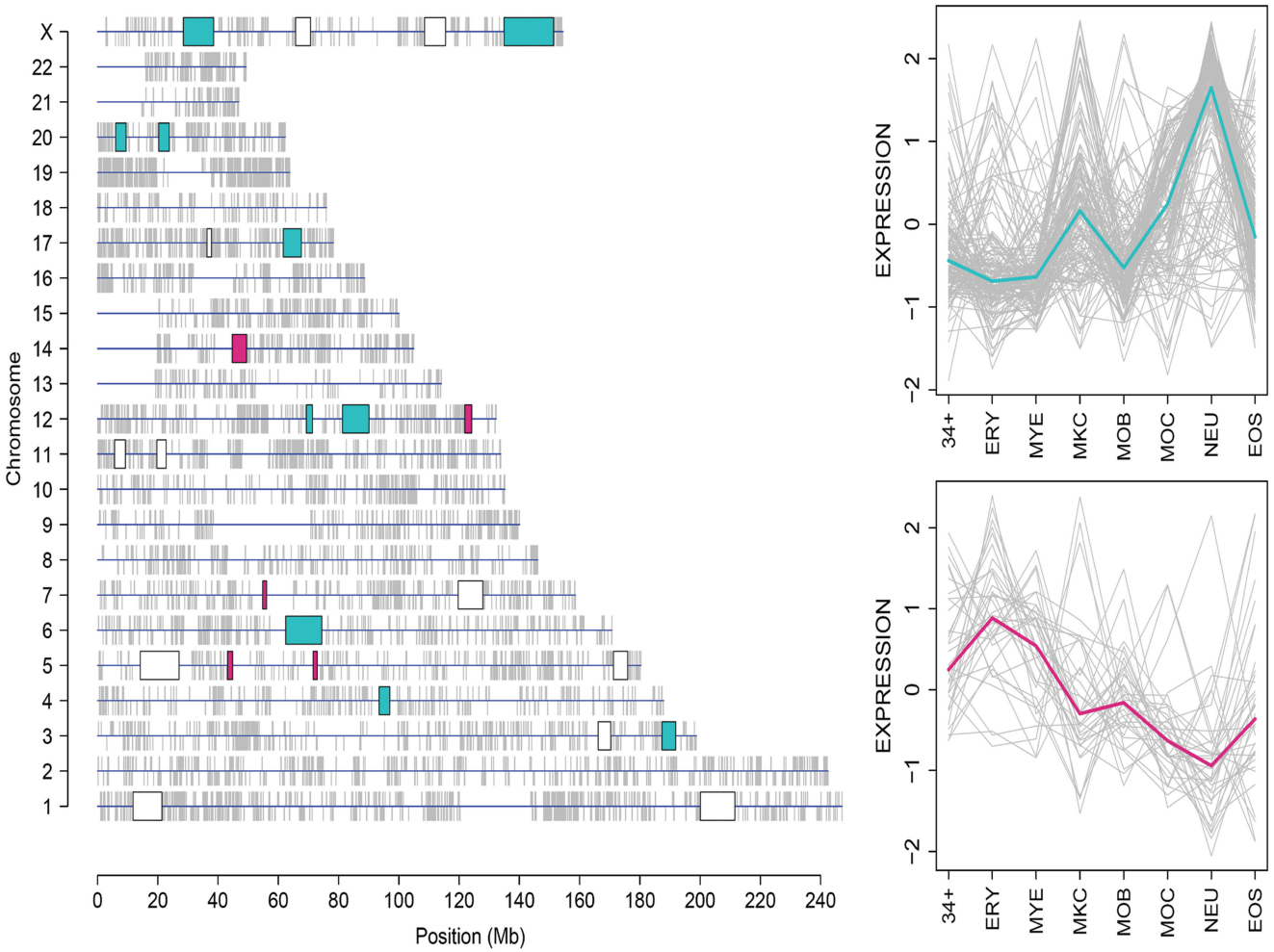

Figure 6. Genomic localization of CEMR. Genomic positions of 26 original positively correlated CER in the human chromosomes and their grouping into CEMR are shown. The two CEMR are marked in magenta and light blue colours both in the chromosomes plot on the left and in the expression profiles on the right. White blocks on the chromosomes plot on the left represent CER which can't be grouped into CEMR according to the selected thresholds used for the analysis. (34+: CD34+ HSC; ERY: Erythroblasts; MYE: Myeloblasts; MKC: Megakaryoblasts; MOB: Monoblasts; MOC: Monocytes; NEU: Neutrophils; EOS: Eosinophils).

similar number of genes, so as to exclude any possible bias related to the number of genes in each gene set because a non-negligible difference exists between the number of genes in CEG (mean 30.2) and CER (mean 15.6). Thus, we focused on 21 CEG and 10 CER (ranging from 15 to 29 genes each) and, after sorting gene sets according to the number of over-represented motifs, we found a significant enrichment in CER among the gene sets with higher number of motifs (9 CER out of the 15 gene sets, *P*-value 0.00187; Figure 8, main panel). Moreover, the number of motifs identified in CEG and CER is significantly different also considering *t*-tests results: *P*-values are significant at $\alpha = 0.05$ both considering all motifs and considering subsets of motifs with specific biological meaningful characteristics (Figure 8, small panel).

Motif discovery results in a pathways-oriented view. KEGG (http://www.genome.ad.jp/kegg) and Biocarta (http://www.biocarta.com) databases provide pathway maps representing knowledge on molecular interactions and networks involved in metabolism as well as genetic or environmental regulation of cellular processes. Ingenuity pathway analysis (IPA; http://www.ingenuity.com) is a commercial software for modeling biological systems. We integrated results of motifs discovery in SELGPi (e.g. CEG sets) with biologically meaningful annotations on gene relationships such as KEGG and Biocarta information, and with IPA-supported charts of functional relationships. In particular, we considered gene sets associated to over-represented motifs possibly recognized by a TF known to have a role in myelopoiesis. These sets of genes, together with the putative regulator(s) (i.e. the known TF binding the discovered motif), were mapped to KEGG and Biocarta pathways, and analysed by IPA. This allowed finding interesting examples of regulators and regulated gene products, which are involved in specific pathways or regulatory circuits.

For instance, CEG5 genes are highly expressed in erythroblasts and megakaryoblasts, and at least in part

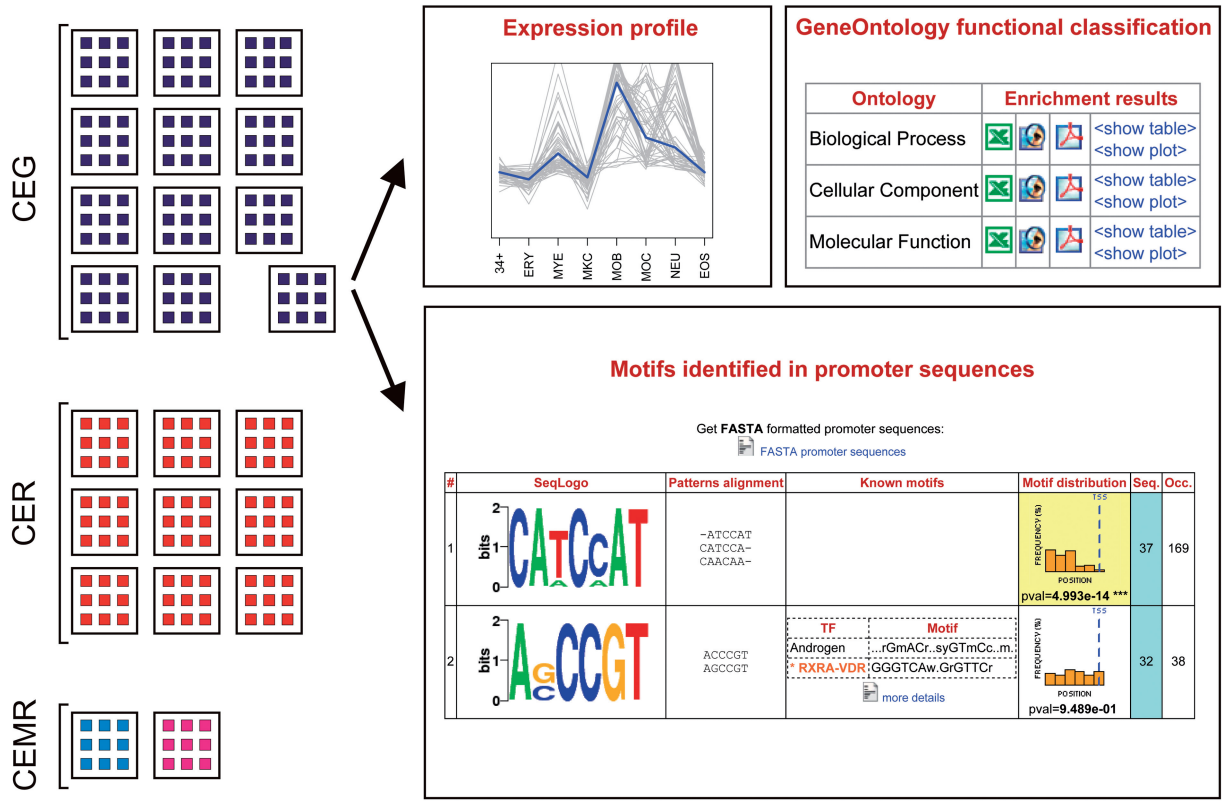

Figure 7. Results of motif discovery analysis. The results of motif discovery analysis on promoters of each selected set of genes are integrated with gene expression data, functional information about genes, and more details concerning motifs characteristics in a dedicated website (http://compgen.bio.unipd.it/MoDi/). The dedicated website allows simultaneous exploration of information concerning the selected sets of genes and the over-represented motifs.

Table 1. Statistics on over-represented motifs

	Genes	Motifs										
		All	Similar to known TFBS		Similar to myeloid TFBS		Non-uniform (NU)		Occurring in highly correlated subgroup of genes (HC)		NU and HC	
	N	*N*	*N*	Percentage	*N*	Percentage	*N*	Percentage	*N*	Percentage	*N*	Percentage
Gene sets (59 out of 72)												
Total	1532.0	5325.0	1009.0		187.0		1772.0		1881.0		578.0	
Average	26.0	90.3	17.1	18.9	3.2	3.5	30.0	33.3	31.9	35.3	9.8	10.9
CEG (32 out of 44)												
Total	967.0	1943.0	367.0		70.0		651.0		645.0		210.0	
Average	30.2	60.7	11.5	18.9	2.2	3.6	20.3	33.5	20.2	33.2	6.6	10.8
CER (25 out of 26)												
Total	390.0	3369.0	637.0		117.0		1109.0		1227.0		359.0	
Average	15.6	134.8	49.0	36.4	4.7	3.5	85.3	63.3	94.4	70.0	27.6	20.5
CEMR (2 out of 2)												
Total	175.0	13.0	5.0		0.0		12.0		9.0		9.0	
Average	87.5	6.5	2.5	38.5	0.0	0.0	6.0	92.3	4.5	69.2	4.5	69.2

The table reports information on the over-represented motifs that where found in the whole set of selected gene sets and in the three distinct types of gene sets (CEG, CER and CEMR). Reported mean values are calculated only considering those gene sets with over-represented motifs: the number of gene sets with over-represented motifs is shown in the first column, together with the total number of gene sets for each group. Then for each group of gene sets, statistics on the total number of genes are shown along with statistics on the total number of over-represented motifs (All), as well as with statistics on the number of over-represented motifs matching specific characteristics: including motifs matching known TFBS (known TFBS), including motifs matching known TFBS with a known role in myelopoiesis, motifs with non-uniform distribution along the promoters (non-uniform), motifs occurring in a subgroup of highly correlated genes and motifs satisfying the latter two characteristics.

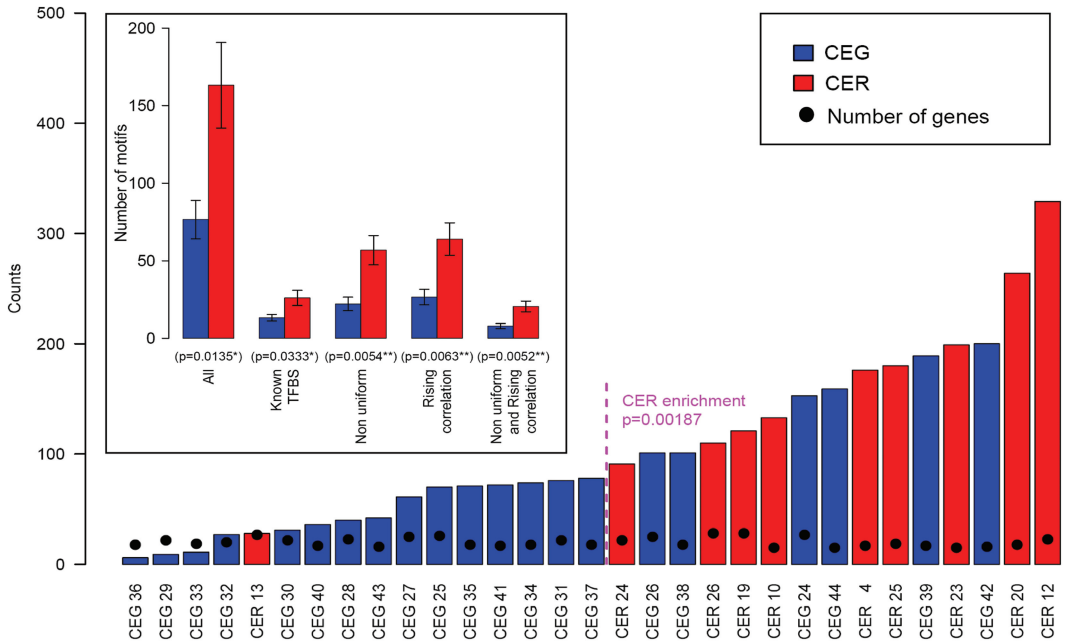

Figure 8. Comparison of the number of significant motifs found in CEG and CER. The number of significantly over-represented motifs within promoters of 21 CEG (blue bars) and 10 CER (red bars) of similar cardinality (from 15 to 29 genes each, as indicated by black points in the main panel) was compared. The significance of the difference was tested with *t*-test by considering all motifs and subgroup of motifs satisfying different characteristics (small panel). In addition, in the main panel, the selected sets of genes are sorted according to the number of over-represented motifs discovered in each gene set. The relative enrichment in CER (9 out of 15), in the first half of gene sets with higher number of motifs, is significant according to hypergeometric test (*P*-value 0.00187).

regulated by NFYA, according to motif discovery results, which are known to be involved in myeloid cells differentiation and to be expressed in erythroid and myeloid progenitors. In addition, many genes included in CEG5 show different types of previously reported interactions, which can be enlightened using the IPA software (Figure 9); this software allows identifying network of relationships among selected sets of genes according to the literature reports. This interaction network between the genes included in CEG5, by means of IPA, constitutes a significant finding and confirms the existence of biologically meaningful relationships, in addition to the sharing of sequence motifs, among the identified genes.

CEG44 genes are highly expressed in megakaryoblasts, and a motif similar to GATA1 binding site is present in half of their promoters. Two of these genes are included into the KEGG pathway 'Arachidonic acid metabolism: prostaglandin and leukotriene metabolism' (Supplementary Data file 7, panel A). Platelets contain thromboxanes that are derived from arachidonic acid and are relevant for platelets function, including their aggregation and activation. The same gene set also includes pro-platelet basic protein (PPBP) that is involved in platelet production and other genes involved in other haematopoietic cells differentiation, such as Bruton agammaglobulinemia tyrosine kinase (BTK), that is also expressed in platelets, and monocyte to macrophage differentiation-associated (MMD).

Finally, CEG33 genes that are characterized by expression peaking in erythroblasts, and the putative regulators GATA1 and LMO2, identified according with motif discovery results, were mapped on Biocarta pathways. GATA1 and AHSP [also named erythroid-associated factor (ERAF)] are included in the gene set and are known to interact in the pathway of the Haemoglobin's Chaperone. This gene set with an erythroid-specific pattern of expression includes as well Glycophorin E, an erythroid antigen related to the M blood group and Adducin-2 (ADD2) that is involved in regulating erythrocytes' precursors proliferation: the figure in panel B of Supplementary Data file 7 shows a simplified version of Haemoglobin's Chaperone chart derived from Biocarta. Furthermore, the gene set includes other genes involved in the differentiation of other haematopoietic cells such as PRKDC (protein kinase, DNA-activated, catalytic polypeptide) that is relevant for B cells differentiation and is also expressed in myeloid cells or DLK1 (delta-like 1 homolog) that is involved in T cells differentiation.

DISCUSSION

In the present work, we considered different levels of gene expression regulation in human myelopoiesis, integrating the study of promoter-based and position-related control of transcription, of ~10 000 genes, in myeloid cells and identifying sequence elements in gene promoters with putative regulatory function along differentiation.

A novel methodology for the identification of putative regulatory elements in promoter sequences was developed, aiming at identifying motifs over-represented in a selected set of promoters, as compared with a background model built according to a reference set of promoters.

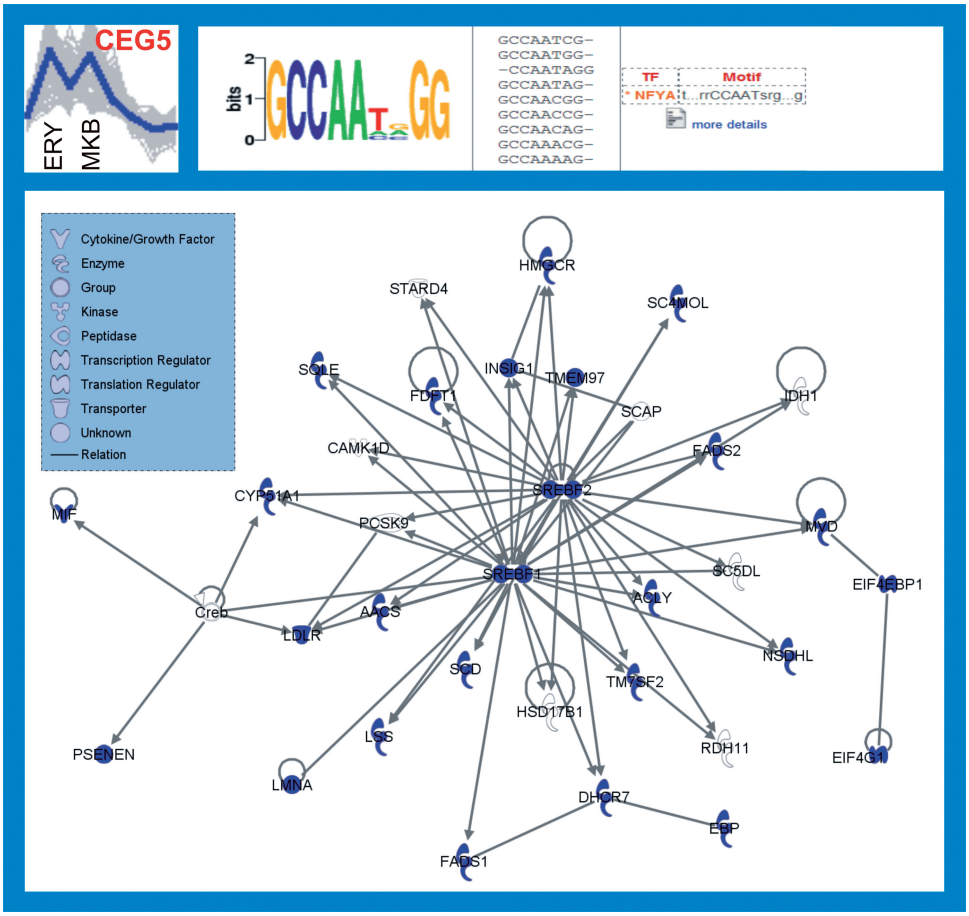

Figure 9. Expression profile, example of motif discovery results and network of interactions between genes included in CEG5. The top left panel reports expression plot for CEG5 genes, with marked expression peaks corresponding to erythroblasts and megakaryoblasts cell contexts. The top right panel contains a screenshot from the website with supplementary results (http://compgen.bio.unipd.it/MoDi/) showing one of the over-represented sequence motifs matching a known binding site for NFYA. In details, this panel reports the sequence logo of the discovered motif; the sequences are composed of the discovered motif and the known motif associated to NFYA binding site. The bottom panel represents the network of interactions obtained from IPA analysis on the CEG5 genes. Within the network, nodes represent gene products, whereas edges represent biologically meaningful interactions supported by literature findings. Shapes of different nodes represent different molecular functions, as detailed in the legend. Arcs (arrows) represent regulatory relationships (gene product A activates expression of gene B or regulates the activity of protein B), whereas edges represent protein–protein interactions.

Different lines of evidence support the fact that promoter regulatory elements are functional in a given biological context according to their position relative to the TSS. Tabach and colleagues (20) proved that positional-dependent TFBS tend to be located in the region close to and upstream the TSS. Thus, promoter sequences were divided in partially overlapping windows, which were considered individually for the selection of over-represented patterns.

The framework for the identification of regulatory motifs integrates a number of steps, including approximate patterns enumeration, calculation of exact patterns over-representation, and generation of motifs by clustering sequences of exact patterns. The first step of the analysis is the enumeration of approximate patterns found in the selected group of promoters. Biologically, functional binding sites are degenerated and the over-representation of exact patterns is a subtle signal, often too difficult to identify with statistical techniques. The adopted statistical measure that was described by Van Helden *et al.* (28) was based on the binomial distribution, allowing associating a *P*-value to the observed occurrences of patterns or motifs. We adopted the FDR to guarantee a global control over false positives. One improvement of our framework relies on the fact that the over-representation significance was calculated neither for a single exact pattern, nor for a simple approximate one. Instead, we considered the subgroup of exact patterns matching a given approximate one, capable of maximizing the significance of global over-representation of the group in the selected set of gene promoters, as compared with the background model. This approach was finally chosen considering that: (i) the over-representation signal of each exact pattern is subtle and strongly dependent from expected number of occurrences, in turn dependent from pattern length; (ii) the over-representation of a pattern including fully variable positions is not informative because it may match exact patterns both over- and under-represented,

the latter obscuring likely biologically meaningful signals. In the last phase of the analytical pipeline, clustering of all exact patterns belonging to over-represented groups gives rise to over-represented motifs, which are eventually compared with known TFBS and further analysed. Thus, identified motifs are associated to different attributes, such as non-uniform distribution along promoters, accounting for position-dependent function, and occurrence in promoters of highly CEG, possibly indicating that the presence of the motifs is crucial for determining specific expression behaviour. Non-uniformly distributed motifs occurring in promoters of highly CEG are probably the most interesting candidates for a functional role along myelopoiesis.

A very large set of putative promoter sequences (~15 000, i.e. a good representation of the whole set of human promoters) was used as background model for computing patterns over-representation. Using real promoter sequences as background model allows identifying motifs specifically associated to a given set of promoters (of genes sharing expression, positional or functional characteristics) avoiding motifs that are simply highly frequent in human promoter regions. This approach reduces the number of false positives or uninteresting results, i.e. repetitive sequence elements and structural elements constitutively present in most human promoters.

Following a classic assumption that 'genes co-expression implies, at least in part, co-regulation', 44 groups of promoters of highly CEG were identified and associated each to a specific expression pattern in myeloid cells. Then, combining expression and positional information, chromosomal regions with significantly high positive LCS were identified (CER), comprising ~10% of the genes and 7% of the genome. These could represent functional domains of high-level gene expression regulation.

It is worthwhile noting that regions including genes with negative correlation of expression are fewer and smaller than positively correlated regions. Moreover, an apparent inverse correlation exists between the distance of adjacent gene pairs and the correlation coefficient of corresponding expression vectors. Confirming the previous reports (36), in the whole human genome, genes positively correlated tend to be close to each other, whereas negatively correlated genes tend to be separated by larger intergenic regions.

The results of the motifs discovery analyses are available in a dedicated website representing the first collection of putative regulatory motifs acting during myeloid cells differentiation. Expression profiles of genes belonging to a specific gene set are displayed together with information regarding gene annotation, gene-ontology, and promoter sequences. Motifs found in the selected gene set are shown together with all the information on attributes derived from the post-processing of motif discovery results. Results about CERs and CEMRs are also available as a resource based on the DAS, and can be accessed by querying the MyDas server. This represents an expandable framework in which additional results of novel analyses on extended myeloid cells datasets may be easily integrated.

Significantly, over-represented motifs were identified in 80% of considered gene sets, with 90 motifs per gene set in average. About 20% of over-represented motifs are similar to known TFBS, and all of the considered motifs binding TF, with a known role in myeloid cells differentiation, were found over-represented in at least one gene set. About one-third of identified motifs are non-uniformly distributed along promoters and another one-third is represented in promoters of genes with highly correlated expression profiles; the intersection of these categories accounts for 11% of all over-represented motifs.

Motifs discovery results detailed in the website could be profitably used to gain biological interpretation since the various characteristics of each motif are shown together with gene expression and function information. For example, focusing on CEGs, with at least one motif non-uniformly distributed and occurring in a subset of genes with high correlation, it could be noticed that in the promoters of CEG5 three over-represented motifs were found, all similar to the known binding site of nuclear transcription factor Y alpha (NFYA), which was previously reported to be involved in myeloid cells differentiation and to be expressed in erythroid and myeloid progenitors (37–39). The expression pattern of CEG5, peaking in erythroblasts, and megakaryoblasts is coherent with previous reports. In addition, the analysis of Gene Ontology functional classes, and IPA analysis also highlighted the existence of biologically meaningful relationships among CEG5 genes as well as the enrichment in biological process categories related to the synthesis of thromboxanes, and therefore, essential for platelets function. Furthermore, CEG11 shows the highest expression level in monoblasts, and is enriched in genes involved in immune, defence and inflammatory response, as inferred from Gene Ontology functional classification. Among its over-represented motifs that are as well non-uniformly distributed and occurring in highly CEG, we found a motif similar to the binding site for CEBPB, i.e. a well known transcription factor playing a role in the differentiation and functionality of mono/macrophages and also granulocytes (40–42). From another point of view, motifs discovery results can help identifying possible regulatory circuits in myeloid differentiation, involving specific transcription factors and/or combination of them. For example, GATA1 (GATA binding protein 1/globin transcription factor 1) has an established role in the differentiation of megakaryocytes, erythrocytes and also eosinophils (43,44). Motifs possibly recognized by GATA1 are present among over-represented motifs of CEG33 and CEG42, including genes with, respectively, erythroblasts- and eosinophils-specific expression patterns, as well as among motifs of CEG34 and CEG44, comprising genes with megakaryoblasts-specific expression patterns. Moreover, among these gene sets, CEG33 and CEG44 share predicted binding sequences for LMO2 (LIM domain only 2), a transcription factor with a role in haematopoiesis, with previously described interactions with GATA1 (45). In particular, it can interact with GATA1 and is involved in erythroid differentiation (43,46). It is worth noticing that CEG44 includes a limited number of genes, and therefore over-represented Gene Ontology functional classes cannot be identified with statistical significance. Nevertheless, the mapping of CEG44 genes on KEGG pathways and additional functional information

allowed enlightening biologically meaningful relationships between the selected genes. Then, focusing on SPI1 (spleen focus forming virus (SFFV) proviral integration oncogene spi1) (43), which has a role in influencing myeloid cells differentiation towards granulocytes (neutrophils), monocytes and also eosinophils cell lineages, we found over-represented motifs corresponding to its known-binding sites in many CEG and CER gene sets. Among them, there are CEG20, CEG24 and CEG42, with an eosinophils-specific expression pattern, as well as different CER including genes with high expression in neutrophils (such as CER3, CER4, CER5, CER15, CER20, CER22 and CER24). Furthermore, these CER are enriched in genes involved in development and immune system related functional categories according to the analysis of Gene Ontology functional classes. In addition, motif discovery results were integrated with biologically relevant information concerning regulatory and metabolic pathways as well as with databases describing molecular interactions such as IPA. The integration of computational results and biological data allowed verifying that complex relationships can be enlightened in the selected gene sets as described in the Results section.

When observing the variety of expression profiles associated to the different CEG sets and to positive CER gene sets, it is evident that the CER profiles can be fitted to a smaller number of expression patterns, showing peculiar expression behaviours in differentiation lineages, and that their cross comparison allows the identification of only two groups of CER gene sets, i.e. the CEMR. These findings allow supposing that mechanisms controlling expression of specific chromosomal regions, which may involve epigenetic modifications, could be particularly important in specific differentiation lineages. The expression patterns of the two CEMR can indeed be related to cell contexts representing early and late differentiation stages, and gene co-expression in CER and CEMR gene sets might be related to different levels of epigenetic regulation.

Genes adjacent each other on a chromosome can be under the influence of the same, locally acting, regulators and/or under the effect of local epigenetic control, based on specific chromatin modifications. Moreover, additional levels of epigenetic regulation may act on genes located on a given functional district of the three-dimensional interphase nucleus (26,34). Indeed, the resultant gene expression derives from different layers of transcriptional regulation, mediated by epigenetic mechanisms and by specific combinations of transcription factors binding sequences, within gene promoters. In this view, we performed the comparative study of promoters of CEG, CER and CEMR with the final purposes of identifying putatively functional regulatory elements, involved in specific differentiation switches and lineage choices in myeloid cells, and formulating hypotheses on the relative role of genetic and epigenetic regulation of transcription during myelopoiesis.

More motifs were found in the groups of promoters of CER gene sets than those of promoters of CEG with comparable cardinality. However, since CERs share basically two expression profiles, we wondered if the total number of identified motifs in CER gene sets could have been overestimated and if the same motifs were actually found over-represented, by parallel analyses, in different CER gene sets. To assess whether the higher number of motifs found in CERs was due to a high number of shared motifs between CERs, we built a list of non-redundant motifs found in the two supersets containing all results of CERs and all CEGs by pairwise comparison of motifs IUPAC consensus sequences. In the two supersets, non-redundant motifs were 16% fewer than original motifs (1653 and 2754 non-redundant motifs were identified in CEG and CER, respectively) and the ratio between motifs found in CER and CEG gene sets remains unchanged if non-redundant motifs are considered. Thus, conversely, the number of non-redundant motifs found over-represented in CER gene promoters remains considerably higher than that found in CEG gene promoters. This result is in accordance with the fact that few significantly over-represented motifs were found in CEMR gene sets: the gene promoters of different CER sets, grouped by similarity of expression in a CEMR set, do not share numerous similar motifs.

The motifs discovery approach is characterized by the independent analysis of different promoter regions, and by the posterior analysis of distribution of over-represented motifs, accounting for the role of binding site position in determining its function. The identification of motifs found in subgroups of genes highly co-expressed and comparison of detected motifs with known TFBS is expected to increase the completeness of results. The statistical analysis adopted for identifying putative biologically relevant motifs was both stringent, adopting false discovery rate, and capable of identifying subtle over-representation signals. Indeed, the analysis is conducted on subgroups of exact patterns for which the significance of global over-representation is maximized in the selected set of gene promoters, as compared with the background model based on a numerous set of real gene promoter sequences.

In conclusion, the integrated analysis of co-expressed and/or co-localized sets of genes proved to be effective in enlightening biologically relevant results, including findings related to the differentiation of various myeloid lineages, which are coherent with previous knowledge of the considered, complex biological system. This work constitutes an important improvement in the methodologies for characterizing gene expression regulation of entire genomic regions biologically relevant for a specific process.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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REFERENCES

1. Kosak,S.T., Scalzo,D., Alworth,S.V., Li,F., Palmer,S., Enver,T., Lee,J.S. and Groudine,M. (2007) Coordinate gene regulation during hematopoiesis is related to genomic organization. *PLoS. Biol.*, **5**, e309.
2. Caron,H., van Schaik,B., van der,M.M., Baas,F., Riggins,G., van Sluis,P., Hermus,M.C., van Asperen,R., Boon,K., Voute,P.A. *et al.* (2001) The human transcriptome map: clustering of highly expressed genes in chromosomal domains. *Science*, **291**, 1289–1292.
3. Lercher,M.J., Urrutia,A.O. and Hurst,L.D. (2002) Clustering of housekeeping genes provides a unified model of gene order in the human genome. *Nat. Genet.*, **31**, 180–183.
4. Vogel,J.H., von Heydebreck,A., Purmann,A. and Sperling,S. (2005) Chromosomal clustering of a human transcriptome reveals regulatory background. *BMC Bioinformatics*, **6**, 230.
5. Purmann,A., Toedling,J., Schueler,M., Carninci,P., Lehrach,H., Hayashizaki,Y., Huber,W. and Sperling,S. (2007) Genomic organization of transcriptomes in mammals: coregulation and cofunctionality. *Genomics*, **89**, 580–587.
6. Coppe,A., Danieli,G.A. and Bortoluzzi,S. (2006) REEF: searching REgionally Enriched Features in genomes. *BMC Bioinformatics*, **7**, 453.
7. Michalak,P. (2008) Coexpression, coregulation, and cofunctionality of neighboring genes in eukaryotic genomes. *Genomics*, **91**, 243–248.
8. Ferrari,F., Bortoluzzi,S., Coppe,A., Basso,D., Bicciato,S., Zini,R., Gemelli,C., Danieli,G.A. and Ferrari,S. (2007) Genomic expression during human myelopoiesis. *BMC Genomics*, **8**, 264.
9. Hertz,G.Z. and Stormo,G.D. (1999) Identifying DNA and protein patterns with statistically significant alignments of multiple sequences. *Bioinformatics*, **15**, 563–577.
10. Hughes,J.D., Estep,P.W., Tavazoie,S. and Church,G.M. (2000) Computational identification of cis-regulatory elements associated with groups of functionally related genes in Saccharomyces cerevisiae. *J. Mol. Biol.*, **296**, 1205–1214.
11. Pevzner,P.A. and Sze,S.H. (2000) Combinatorial approaches to finding subtle signals in DNA sequences. *Proc. Int. Conf. Intell. Syst. Mol. Biol.*, **8**, 269–278.
12. Bailey,T.L. and Elkan,C. (1995) The value of prior knowledge in discovering motifs with MEME. *Proc. Int. Conf. Intell. Syst. Mol. Biol.*, **3**, 21–29.
13. Thompson,W., Rouchka,E.C. and Lawrence,C.E. (2003) Gibbs Recursive Sampler: finding transcription factor binding sites. *Nucleic Acids Res.*, **31**, 3580–3585.
14. Pavesi,G., Mereghetti,P., Mauri,G. and Pesole,G. (2004) Weeder Web: discovery of transcription factor binding sites in a set of sequences from co-regulated genes. *Nucleic Acids Res.*, **32**, W199–W203.
15. Wang,G., Yu,T. and Zhang,W. (2005) WordSpy: identifying transcription factor binding motifs by building a dictionary and learning a grammar. *Nucleic Acids Res.*, **33**, W412–W416.
16. Bortoluzzi,S., Coppe,A., Bisognin,A., Pizzi,C. and Danieli,G.A. (2005) A multistep bioinformatic approach detects putative regulatory elements in gene promoters. *BMC Bioinformatics*, **6**, 121.
17. Pizzi,C., Bortoluzzi,S., Bisognin,A., Coppe,A. and Danieli,G.A. (2005) Detecting seeded motifs in DNA sequences. *Nucleic Acids Res.*, **33**, e135.
18. Thomas-Chollier,M., Sand,O., Turatsinze,J.V., Janky,R., Defrance,M., Vervisch,E., Brohee,S. and van Helden,J. (2008) RSAT: regulatory sequence analysis tools. *Nucleic Acids Res.*
19. Tompa,M., Li,N., Bailey,T.L., Church,G.M., De Moor,B., Eskin,E., Favorov,A.V., Frith,M.C., Fu,Y., Kent,W.J. *et al.* (2005) Assessing computational tools for the discovery of transcription factor binding sites. *Nat. Biotechnol.*, **23**, 137–144.
20. Tabach,Y., Brosh,R., Buganim,Y., Reiner,A., Zuk,O., Yitzhaky,A., Koudritsky,M., Rotter,V. and Domany,E. (2007) Wide-scale analysis of human functional transcription factor binding reveals a strong bias towards the transcription start site. *PLoS. ONE.*, **2**, e807.
21. Casimiro,A.C., Vinga,S., Freitas,A.T. and Oliveira,A.L. (2008) An analysis of the positional distribution of DNA motifs in promoter regions and its biological relevance. *BMC Bioinformatics*, **9**, 89.
22. Ferrari,F., Bortoluzzi,S., Coppe,A., Sirota,A., Safran,M., Shmoish,M., Ferrari,S., Lancet,D., Danieli,G.A. and Bicciato,S. (2007) Novel definition files for human GeneChips based on GeneAnnot. *BMC Bioinformatics*, **8**, 446.
23. Schug,J., Schuller,W.P., Kappen,C., Salbaum,J.M., Bucan,M. and Stoeckert,C.J. Jr (2005) Promoter features related to tissue specificity as measured by Shannon entropy. *Genome Biol.*, **6**, R33.
24. Heyer,L.J., Kruglyak,S. and Yooseph,S. (1999) Exploring expression data: identification and analysis of coexpressed genes. *Genome Res.*, **9**, 1106–1115.
25. Callegaro,A., Basso,D. and Bicciato,S. (2006) A locally adaptive statistical procedure (LAP) to identify differentially expressed chromosomal regions. *Bioinformatics*, **22**, 2658–2666.
26. Cremer,T., Cremer,M., Dietzel,S., Muller,S., Solovei,I. and Fakan,S. (2006) Chromosome territories – a functional nuclear landscape. *Curr. Opin. Cell Biol.*, **18**, 307–316.
27. Brazma,A., Jonassen,I., Vilo,J. and Ukkonen,E. (1998) Predicting gene regulatory elements in silico on a genomic scale. *Genome Res.*, **8**, 1202–1215.
28. van Helden,J., Andre,B. and Collado-Vides,J. (1998) Extracting regulatory sites from the upstream region of yeast genes by computational analysis of oligonucleotide frequencies. *J. Mol. Biol.*, **281**, 827–842.
29. Benjamini,Y. and Hochberg,Y. (1995) Controlling the false discovery rate – a practical and powerful approach to multiple testing. *J. Roy. Stat. Soc. Ser. B – Methodol.*, **57**, 289–300.
30. Hastie,T., Tibshirani,R. and Friedman,J. (2001) *The Elements of Statistical Learning; DataMining, Inference and Prediction*. Springer, New York.
31. Gordon,D.B., Nekludova,L., McCallum,S. and Fraenkel,E. (2005) TAMO: a flexible, object-oriented framework for analyzing transcriptional regulation using DNA-sequence motifs. *Bioinformatics*, **21**, 3164–3165.
32. Thompson,J.D., Higgins,D.G. and Gibson,T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.*, **22**, 4673–4680.
33. Dowell,R.D., Jokerst,R.M., Day,A., Eddy,S.R. and Stein,L. (2001) The distributed annotation system. *BMC Bioinformatics*, **2**, 7.
34. Meaburn,K.J. and Misteli,T. (2007) Cell biology: chromosome territories. *Nature*, **445**, 379–781.
35. Trinklein,N.D., Aldred,S.F., Hartman,S.J., Schroeder,D.I., Otillar,R.P. and Myers,R.M. (2004) An abundance of bidirectional promoters in the human genome. *Genome Res.*, **14**, 62–66.
36. Fukuoka,Y., Inaoka,H. and Kohane,I.S. (2004) Inter-species differences of co-expression of neighboring genes in eukaryotic genomes. *BMC Genomics*, **5**, 4.
37. Marziali,G., Perrotti,E., Ilari,R., Coccia,E.M., Mantovani,R., Testa,U. and Battistini,A. (1999) The activity of the CCAAT-box binding factor NF-Y is modulated through the regulated expression of its A subunit during monocyte to macrophage differentiation: regulation of tissue-specific genes through a ubiquitous transcription factor. *Blood*, **93**, 519–526.
38. Sjin,R.M., Krishnaraju,K., Hoffman,B. and Liebermann,D.A. (2002) Transcriptional regulation of myeloid differentiation primary response (MyD) genes during myeloid differentiation is mediated by nuclear factor Y. *Blood*, **100**, 80–88.
39. Miller,J.D., Stacy,T., Liu,P.P. and Speck,N.A. (2001) Core-binding factor beta (CBFbeta), but not CBFbeta-smooth muscle myosin

heavy chain, rescues definitive hematopoiesis in CBFbeta-deficient embryonic stem cells. *Blood*, **97**, 2248–2256.
40. Pham,T.H., Langmann,S., Schwarzfischer,L., El Chartouni,C., Lichtinger,M., Klug,M., Krause,S.W. and Rehli,M. (2007) CCAAT enhancer-binding protein beta regulates constitutive gene expression during late stages of monocyte to macrophage differentiation. *J. Biol. Chem.*, **282**, 21924–21933.
41. Duprez,E., Wagner,K., Koch,H. and Tenen,D.G. (2003) C/EBPbeta: a major PML-RARA-responsive gene in retinoic acid-induced differentiation of APL cells. *EMBO J.*, **22**, 5806–5816.
42. Nerlov,C., McNagny,K.M., Doderlein,G., Kowenz-Leutz,E. and Graf,T. (1998) Distinct C/EBP functions are required for eosinophil lineage commitment and maturation. *Genes Dev.*, **12**, 2413–2423.
43. Cantor,A.B. and Orkin,S.H. (2002) Transcriptional regulation of erythropoiesis: an affair involving multiple partners. *Oncogene*, **21**, 3368–3376.
44. Rekhtman,N., Radparvar,F., Evans,T. and Skoultchi,A.I. (1999) Direct interaction of hematopoietic transcription factors PU.1 and GATA-1: functional antagonism in erythroid cells. *Genes Dev.*, **13**, 1398–1411.
45. Osada,H., Grutz,G., Axelson,H., Forster,A. and Rabbitts,T.H. (1995) Association of erythroid transcription factors: complexes involving the LIM protein RBTN2 and the zinc-finger protein GATA1. *Proc. Natl Acad. Sci. USA*, **92**, 9585–9589.
46. Perry,C. and Soreq,H. (2002) Transcriptional regulation of erythropoiesis. Fine tuning of combinatorial multi-domain elements. *Eur. J. Biochem.*, **269**, 3607–3618.