the findings of Hofmann et al.[12] Our results do not rule out a more important role for TRPC1 in the SOCE activity of other cells (Hassock et al[3]) where the subunit composition of the TRP channels need to be determined. Rosado and Sage[4,5] have used the anti-TRPC1 antibody from Alomone. The manufacturer's website (http://www.alomone.com/Site/p_home/home.htm) states that in rat brain it recognizes 2 products: a protein larger than 250 kDa and more faintly a protein at approximately 120 kDa. The molecular size of hTRPC1β is 80 kDa and the full length isoform is 34 amino acids longer.[3] Recently Ong et al[13] showed that the Alomone antibody did recognize a 120 kDa protein in mouse liver and mouse brain. However, it did not recognize overexpressed hTRPC1 under conditions in which a number of other antibodies did, including the anti-XTRP1 antibody used in our study. Therefore the identity of the proteins recognized by the Alomone antibody needs to be clarified. Using the Alomone anti-TRPC1 antibody, we have been unable to reproduce the findings of Rosado and Sage,[4,5] although we cannot rule out variations of antibody specificity between different batches supplied by the manufacturer. We therefore suggest that results obtained with the Alomone anti-TRPC1 antibody should be verified with other better-established antibodies.

In conclusion, we feel that our work represents an important advance in our knowledge of the expression and role of TRPC proteins in platelet Ca^{2+} homeostasis. We have demonstrated the expression of TRPC6 and its role as a SOCE-independent Ca^{2+} entry channel in platelets. Clearly much remains to be determined regarding the molecules and mechanisms involved with the SOCE pathway in platelets.

Kalwant S. Authi, Sheila Hassock, Michael X. Zhu, Veit Flockerzi, and Claudia Trost

Correspondence: Kalwant Authi, King's College London, Centre for Cardiovascular Biology and Medicine, New Hunt's House, Guy's Campus, London, SE1 1UL, United Kingdom; e-mail: kalwant.authi@kcl.ac.uk

References

1. Putney JW Jr, Broad LM, Braun FJ, Lievremont JP, Bird GS. Mechanisms of capacitative calcium entry. J Cell Sci. 2001;114:2223-2229.
2. Clapham DE, Runnels LW, Strubing C. The TRP ion channel family. Nat Rev Neurosci. 2001;2:387-396.
3. Hassock S, Zhu MX, Trost C, Flockerzi V, Authi KS. Expression and role of TRPC proteins in human platelets: evidence that TRPC6 forms the store-independent calcium entry channel. Blood. 2002;100:2801-2811.
4. Rosado JA, Sage SO. Coupling between inositol 1,4,5-trisphosphate receptors and human transient receptor potential channel 1 when intracellular Ca^{2+} stores are depleted. Biochem J. 2000;350:631-635.
5. Rosado JA, Sage SO. Activation of store-mediated calcium entry by secretion-like coupling between the inositol 1,4,5-trisphosphate receptor type II and human transient receptor potential (hTrp1) channels in human platelets. Biochem J. 2001;356:191-198.
6. Zavoico GB, Halenda SP, Sha'afi RI, Feinstein MB. Phorbol myristate acetate inhibits thrombin-stimulated Ca^{2+} mobilization and phosphatidylinositol 4,5-bisphosphate hydrolysis in human platelets. Proc Natl Acad Sci U S A. 1985; 82:3859-3862.
7. Hofmann T, Obukhov AG, Schaefer M, Harteneck C, Gudermann T, Schultz G. Direct activation of human TRPC6 and TRPC3 channels by diacylglycerol. Nature. 1999;397:259-263.
8. Rosado JA, Sage SO. Protein kinase C activates non-capacitative calcium entry in human platelets. J Physiol. 2000;529:159-169.
9. Venkatachalam K, Ma HT, Ford DL, Gill DL. Expression of functional receptor-coupled TRPC3 channels in DT40 triple receptor $InsP_3$ knockout cells. J Biol Chem. 2001;276:33980-33985.
10. Jenner S, Farndale RW, Sage SO. The effect of calcium-store depletion and refilling with various bivalent cations on tyrosine phosphorylation and Mn^{2+} entry in fura-2-loaded human platelets. Biochem J. 1994;303:337-339.
11. Mori Y, Wakamori M, Miyakawa T, et al. Transient receptor potential 1 regulates capacitative Ca^{2+} entry and Ca^{2+} release from endoplasmic reticulum in B lymphocytes. J Exp Med. 2002;195:673-681.
12. Hofmann T, Schaefer M, Schultz G, Gudermann T. Subunit composition of mammalian transient receptor potential channels in living cells. Proc Natl Acad Sci U S A. 2002;99:7461-7466.
13. Ong HL, Chen J, Chataway T, et al. Specific detection of the endogenous transient receptor potential (TRP)-1 protein in liver and airway smooth muscle cells using immunoprecipitation and Western-blot analysis. Biochem J. 2002;364:641-648.

To the editor:

A novel *MLL/AF4* fusion gene lacking the *AF4* transactivating domain in infant acute lymphoblastic leukemia

In a 5-month-old child affected by acute lymphoblastic leukemia (ALL) cytogenetic analysis showed a translocation involving chromosomes 4q13, 11q23, and 17q11. Standard reverse transcriptase–polymerase chain reaction (RT-PCR) analysis of t(4;11) breakpoints did not amplify any known *MLL/AF4* mRNA junction. Therefore, we performed Southern analysis of the *MLL* locus in DNA from bone marrow cells sampled at diagnosis and found a rearrangement at locus *MLL* of chromosome 11q23. Panhandle PCR amplification[1] of the fragment comprising the der(11) translocation breakpoints yielded a 4.6-kb fragment corresponding to the *Bam*HI-rearranged fragment detected by Southern analysis. The amplified fragment contained sequences corresponding to *MLL* exon 9 and, after an $AluJ_0$ sequence, a unique nonrepetitive sequence corresponding to the 937 bp of exon 11 of the *AF4* gene. These data indicated a hitherto unknown junction between *MLL* intron 9 and *AF4* intron 10 on der(11).

Because the MLL partner fragment included a sequence homologous to *AF4* exon 11, we designed a new antisense primer on this exon to amplify the junction sequences of *MLL/AF4* cDNA, transcribed from the new *MLL/AF4* fusion gene. RT-PCR analysis, with the primer couple 5′-TTCCCAAAACCACTCCTAGTGA-3′ (sense-*MLL* exon 9) and 5′-TCAGAATGCTCCTGACTCGTG-3′ (antisense-*AF4* exon 11), yielded a 380-bp (predicted) fragment. Sequence analysis of the amplified product confirmed a hybrid mRNA with an in-frame junction between *MLL* exon 9 and *AF4* exon 11 (Figure 1A).

The predicted structure of the new *MLL/AF4* chimeric protein lacks the transcription activation *MLL* motif at residues 2829 to 2883 and the whole *AF4* domain spanning residues 480 to 560, which is encoded by exon 10 of the *AF4* gene and the first nucleotides of exon 11 (Figure 1B).

The *AF4* gene partners the *MLL* gene in 11q23 translocations in about 50% of childhood and adult ALL cases.[2] The *MLL* gene is fused to at least 25 different gene partners. The sequence and the predicted structure of the proteins encoded by these genes do not appear to have any unifying characteristic that would clarify their role in the leukemogenic process. In addition, in some acute leukemias *MLL* may show exon duplications. These issues raised

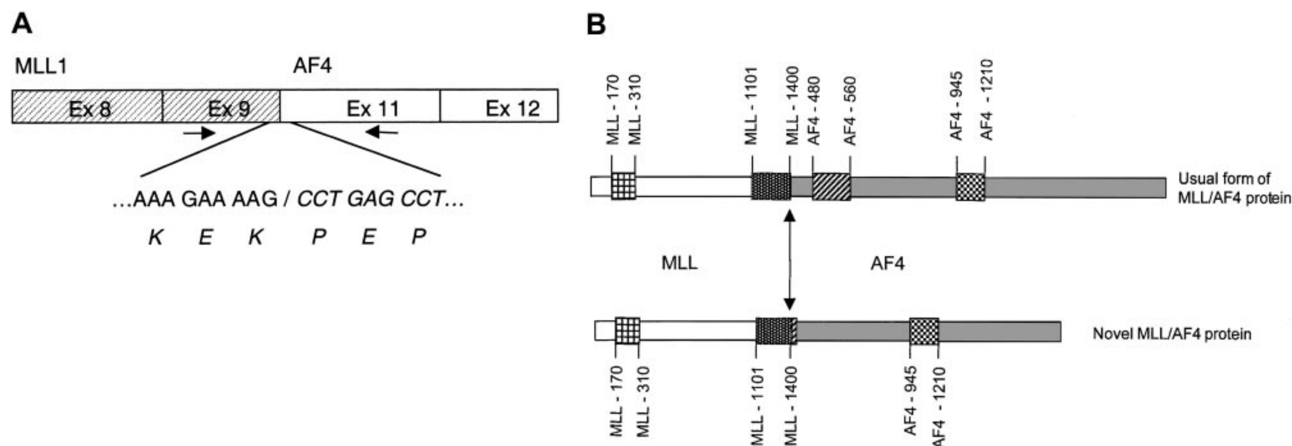

Figure 1. Structural organization of the novel chimeric *MLL/AF4* transcript and protein. (A) Analysis of the novel *MLL/AF4* transcript. An RT-PCR assay was performed to evaluate whether the novel *MLL/AF4* fusion gene generates a chimeric mRNA. cDNA prepared from a bone marrow RNA sample was amplified using the new primers according to sequence analysis of the panhandle product. The amplification reactions consisted of 40 cycles: 94°C, 30 seconds; 65°C, 60 seconds; and 72°C, 60 seconds. (B) Structural organization of the novel chimeric *MLL/AF4* protein. The novel chimeric *MLL/AF4* protein retains the DNA binding domains but lacks the *AF4*-derived transactivating domain, which is conserved in the usual type of this fusion protein. The junctions between the 2 protein sequences are aligned and indicated by an arrow. ▦ indicates *MLL* AT hooks; ▩, *MLL* repression domain; ▨, *AF4* transactivation domain; and ▩, *AF4* nuclear localization.

the possibility that an alteration of *MLL* alone is sufficient to transform hemopoietic precursors and that the fusion partner has no role. However, evidence that partner genes play a role in the leukemogenic process comes from knock-out and knock-in experiments.[3] Indeed, an important role for *AF4* is emerging not only from the epidemiologic restriction of *MLL/AF4* to ALL[4] but also from the recent finding that lymphoid development is severely impaired in *AF4*−/− mice.[3]

The normal function of the *AF4* gene is not known, but a domain with transcriptional activity at nucleotides 480 to 560 and the nuclear localization of the *AF4* protein[5,6] suggests it regulates transcription (ie, the *AF4* gene seems to encode a transcription factor whose expression is relevant for committed lymphoid precursors to complete differentiation). This suggests that the *MLL/AF4* gene arrests the lymphoid differentiation program by altering an early multipotential progenitor cell, either through a gain- or loss-of-function of the *MLL/AF4* protein. Therefore the protein might induce aberrant expression of target genes so impairing lymphoid differentiation,[7] or alternatively it might lose the capacity to induce the expression of genes important for differentiation.[2] The *AF4* breakpoints in this novel *MLL/AF4* fusion gene lie at least 4 introns further downstream compared with all other *MLL/AF4* known translocations. Therefore, the new fusion gene lacks both the *AF4*-derived and the *MLL*-derived (nucleotides 2772-3579) transactivating domain[5] (Figure 1B). The resulting fusion protein is hence able to bind DNA through the *MLL*-derived AT hook domain, and through the *AF4*-derived nuclear targeting sequence domains[8] but could lose its capacity to activate the expression of these genes. Consequently, it is not inconceivable that loss-of-function, through transcription block, could be the mechanism of leukemogenesis in our ALL patient.

It is not clear whether transcription block could apply to other *MLL/AF4* fusion genes that retain the transactivating domain at residues 480 to 560.

Fabrizio Pane, Mariano Intrieri, Barbara Izzo, Concetta Quintarelli, Domenico Vitale, Roberta Migliorati, Lucia Sebastio, and Francesco Salvatore

Correspondence: Fabrizio Pane, CEINGE-Biotecnologie Avanzate and Dipartimento di Biochimica e Biotecnologie Mediche, Università di Napoli Federico II, Via S Pansini 5, 80131 Naples, Italy; e-mail: fabpane@unina.it

Supported by grants from AIRC (Associazione Italiana per la Ricerca sul Cancro, Milan), Consiglio Nazionale Delce Ricerche (CNR)–Progetto Strategico & Progetto Finalizzato (PF) Biotecnologie (Rome), Biogem (Naples), MIUR (Ministero Dell' Istruzione, Dell' Università e Della Ricerca, Rome), AIL (Associazione Italiana Leucemie, Rome), and Regione Campania (Naples).

References

1. Felix CA, Kim CS, Megonigal MD, et al. Panhandle polymerase chain reaction amplifies MLL genomic translocation breakpoint involving unknown partner gene. Blood. 1997;90:4679-4686.
2. Look AT. Oncogenic transcription factors in the human acute leukemias. Science. 1997;278:1059-1064.
3. Isnard P, Core N, Naquet P, Djabali M. Altered lymphoid development in mice deficient for the mAF4 proto-oncogene. Blood. 2000;96:705-710.
4. Biondi A, Cimino G, Pieters R, Pui CH. Biological and therapeutic aspects of infant leukemia. Blood. 2000;96:24-33.
5. Prasad R, Yano T, Sorio C, et al. Domains with transcriptional regulatory activity within the ALL1 and AF4 proteins involved in acute leukemia. Proc Natl Acad Sci U S A. 1995;92:12160-12164.
6. Li Q, Frestedt JL, Kersey JH. AF4 encodes a ubiquitous protein that in both native and MLL-AF4 fusion types localizes to subnuclear compartments. Blood. 1998;92:3841-3847.
7. Ma C, Staudt LM. LAF-4 encodes a lymphoid nuclear protein with transactivation potential that is homologous to AF-4, the gene fused to MLL in t(4;11) leukemias. Blood. 1996;87:734-745.
8. Nakamura T, Alder H, Gu Y, et al. Genes on chromosomes 4, 9, and 19 involved in 11q23 abnormalities in acute leukemia share sequence homology and/or common motifs. Proc Natl Acad Sci U S A. 1993;90:4631-4635.