Identification of Genes Interacting with rnt-1 Through Large-Scale RNAi Screening in Caenorhabditis elegans

Kihoo Lee, Jiwon Shim, Jihyun Lee, and Junho Lee

*Department of Biological Sciences and †Department of Biophysics and Chemical Biology, Seoul National University, Seoul, South Korea 151-742, ‡Department of Medicine, Infectious Diseases Division, Rhode Island Hospital, Warren Alpert Medical School of Brown University, Providence, Rhode Island 02903, and §Department of Molecular, Cell and Developmental Biology, University of California, Los Angeles, California 90095

ABSTRACT Although many critical roles of the RUNX family proteins have already been identified, little attention has been given to how these proteins interact with other factors. Elucidating RUNX protein interactions will help extend our understanding of their roles in normal development and tumorigenesis. In this study, we performed large-scale RNAi screening to identify genes that genetically interact with rnt-1, the sole homolog of RUNX protein in the nematode Caenorhabditis elegans. To this end, we took advantage of the fact that C. elegans can survive a severe loss of RNT-1 function with only mild phenotypes, and we looked for genes that caused a synthetic phenotype in the rnt-1 mutant background. We identified seven genes, three of which (cdk-8, cic-1, and sur-2) are involved in transcription, two of which (pgp-2 and cct-5) are involved in stress response, and two of which (D2045.7 and W09D10.4) are involved in signaling cascades, according to their functional gene ontology terms. We further confirmed that the CDK8-containing mediator complex genetically interacts with RNT-1 by showing that knockdown of each component of the CDK8 mediator complex caused a synthetic phenotype, that is, the exploded intestine through the vulva (Eiv) phenotype, in the rnt-1 mutant background. We also identified a putative target gene, acs-4, which is regulated by the RNT-1 and CDK8 mediator complex. Our results strengthen the notion that the CDK8 mediator complex may also act together with RUNX proteins in mammals.

KEYWORDS RNAi, RUNX, CDK8, mediator, genetic interaction

The RUNX genes encode a family of transcription factors that contain a conserved DNA-binding Runt domain. Roles of RUNX genes in various aspects of animal development, such as the development of hematopoietic stem cells, bone, and neuronal cells, have been genetically elucidated through extensive studies of mutations in RUNX genes (Blyth et al. 2005). For instance, translocations on RUNX1 loci were found to occur most commonly in acute leukemia (Osato et al. 1999; Michaud et al. 2002; Osato 2004). Mutations of RUNX1 in mice also result in abnormal development of cells in nervous system, endothelial cells, and immune cells (Wang et al. 1996). Mutations of RUNX2 in humans induce incomplete development of osteoblasts, which results in cleidocranial dysplasia and osteosarcoma (Banerjee et al. 1997; Werner et al. 1999). Further studies of mutations in mice identified that RUNX2 is critical for maturation of osteoblasts in osteogenesis (Otto et al. 1997; Aberg et al. 2004). Elegant studies in mice revealed that RUNX3 is expressed in multiple tissues, such as endothelial cells in the gastrointestinal tract, T cells, dendritic cells, and neuronal cells, and that RUNX3 has roles in both cell proliferation and differentiation (Bangsow et al. 2001; Fukamachi and Ito 2004). Interestingly, mutations in RUNX1 and RUNX2 showed the tendency of haploinsufficiency not only in mice but also in humans. Familial platelet disorder, which is caused by haploinsufficiency of RUNX1, leads to acute myelogenous leukemia (Song et al. 1999). Cleidocranial dysplasia, which is induced by mutations in RUNX2, was found to be the result of a haploinsufficient mutation on RUNX2 (Mundlos et al. 1997).
It is well-known that a major function of RUNX genes in cells is to regulate the balance between cell proliferation and differentiation (Coffman 2003). Because the decision between cell proliferation and differentiation must reflect the precise cellular environment, the roles and action mechanism of RUNX genes have to be exerted in a context-dependent manner (Braun and Woolard 2009). To reflect precise cellular status, multiple regulations including various splicing forms, overlapped expression of RUNX genes, regulation by upstream activators, numerous RUNX-targeted genes (Cohen 2009), and posttranscriptional regulations (Bae and Lee 2006) were identified for RUNX genes. In addition, RUNX proteins do not act alone, but in conjunction with cofactors; most RUNX proteins form heterodimers with evolutionarily conserved cofactors such as CBFβ. Although rich information on the players that act with RUNX proteins has been obtained in the past, the interacting partners that act transiently, weakly, or indirectly with RUNX proteins have, so far, been overlooked or ignored. Therefore, it would be worth using a genetic model system that allows one to identify genetic interactions of genes by examining the resulting phenotypes.

The nematode Caenorhabditis elegans is a good system for studying genetic interaction of RUNX proteins because there is a sole ortholog of RUNX, rnt-1 (Nam et al. 2002; Lee et al. 2004), and the existing mutations of rnt-1 are not lethal. For example, the genetic interaction of rnt-1 with cki-1, a cyclin-dependent kinase inhibitor, allowed identification of the role of rnt-1 in cell fate commitment in the hypodermal seam cell division, which is similar to the functions of mammalian RUNXs (Nimmo et al. 2005). Also, bro-1, a cofactor of rnt-1, interacts with rnt-1 and its mutation or knockdown affects hypoplasia of hypodermal seam cells (Kagoshima et al. 2007; Shim and Lee 2008). In this study, we screened genes that genetically interact with rnt-1 through a large-scale RNAi screening in the viable rnt-1 mutant allele rnt-1(ok351). We identified seven genes that genetically interact with rnt-1 by observation of the phenotype that is caused by the reduction of both genes’ functions but not by either one alone. We then focused on the components of the CDK8 mediator complex and showed that all of them genetically interact with rnt-1. Disruption of both rnt-1 and a component of the CDK8 mediator complex showed the “exploded intestine through the vulva” phenotype. We demonstrate that act-4 is a putative target gene that is regulated simultaneously by rnt-1 and the CDK8-containing mediator complex.

MATERIALS AND METHODS

Nematode strains
The Bristol N2 strain was used as the wild-type C. elegans strain. The rnt-1(ok351) mutant strain was obtained from the Caenorhabditis Genetics Center (CGC, Minneapolis, MN), and the rnt-1(tm491), rnt-1(tm388), and cdk-8(tm1238) mutant strains were kind gifts from National Bioresources Project (Tokyo, Japan). The rnt-1 cdk-8 double mutants were generated by using standard C. elegans techniques and confirmed by single-worm PCR to identify deletions. All strains were grown at 20°C on standard Nematode growth media plates (Brenner 1974).

Feeding RNAi screening
The RNAi library by J. Ahringer (Cambridge, UK), which covers >80% of C. elegans open reading frames, was used in the screen. We screened genes on chromosomes I and III. All Escherichia coli strains were streaked and cultured with ampicillin on Luria broth. Before worms were fed with E. coli strains, 1 mM IPTG was treated to induce ds RNA transcription of the target genes. The phenotypes were observed in the progeny from the mothers that had been subject to RNAi from their L4 stage. We compared phenotypes of wild-type N2 and rnt-1(ok351) animals after each RNAi and further confirmed the phenotypes with other alleles, rnt-1(tm388) and rnt-1(tm491), at least three times. HT115 bacteria carrying L4440, the plasmid of the empty vector pPD129.36, were used as a control RNAi.

Microscopy and imaging
To detect RNAi-induced phenotypes, worms were paralyzed with 1 mM levamisole and mounted on 5% agar pads. DIC images were observed using a Zeiss Axioplan 2 microscope.

Identification of candidate target genes from database
To find genes whose knockdown causes the exploded intestine through the vulva phenotype, we identified candidate target genes from the WormMart at WormBase homepage using “exploded through vulva” as keywords (http://www.wormbase.org). To select genes that harbor the RNT-1 binding sites, R(T/G)ACCRCRA, in their promoter regions, we identified the numbers and locations of the RNT-1 binding sites in the 2-kb region of the promoters by visual inspection using the CLC sequence viewer 6 (http://www.clcbio.com).

RNA preparation, reverse-transcription, and quantitative real-time PCR analysis
Total RNA of mixed-stage worms was isolated with Trizol reagent (Invitrogen) by freeze-thaw method given in the standard protocol served with the manufacturer’s manual. cDNA was synthesized with RevertAid M-MuLV reverse-transcriptase (Fermentas Canada) using oligo-dT primer and subjected to PCR amplification. Real-time quantitative PCR was performed with BIO-RAD iQ SYBR Green Supermix in iQ5 as described in the manufacturer’s manual. Primers used in quantitative real-time PCR were generated by Primer3 (http://frodo.wi.mit.edu) with default setting. All the results of quantitative real-time PCR were triplicated in each of three independent samples. The act-1 gene was used for normalization. Student t test was used for statistical analyses.

RESULTS AND DISCUSSION

The rationale of this study
Although RUNX genes in mammals such as RUNX1 and RUNX2 are known to be haploinsufficient (Blyth et al. 2005), this seems not to be the case for rnt-1 in C. elegans because even homozygous animals that possess any of the three existing in C. elegans survive and do not show severe phenotypes. It is possible that rnt-1 deletion mutant alleles may not be null mutations, although they delete considerable portions of the coding regions. Larger deletions have not been isolated despite extensive random mutagenesis followed by PCR-based screening (J. H. Ahnn, personal communication), and the phenotype caused by rnt-1 RNAi includes embryonic lethality, which is obviously more severe than that of existing deletion mutations. In addition, there is no chromosomal deficiency available that uncovers the rnt-1 locus, suggesting that rnt-1 in C. elegans may be haploinsufficient and that the three existing rnt-1 deletion mutants are not null, but rather reduction-of-function mutations. Consistently, we were able to detect rnt-1 transcripts RT-PCR even though they were produced as truncated transcripts (Figure 1A, bottom panel). Reduction-of-function mutations are not the best resources for genetics because they make it difficult to determine the exact function of a gene. However, they
can sometimes serve as precious tools for elucidating genetic interactions, which otherwise could not be identified because of lethality. We reasoned that the existing deletion mutations of \textit{rnt-1} provide a sensitized genetic background so that one could identify interacting genes by looking for synthetic phenotypes.

**Identification of genes that interact with \textit{rnt-1} by RNAi screening for synthetic phenotypes**

Although the \textit{rnt-1(ok351)} allele has the largest deletion within the coding region, it causes the mildest phenotype among the deletion mutants, that is, a slightly short body length and delayed development. We therefore decided to use \textit{ok351} allele as our sensitized genetic background for synthetic phenotype screening. To isolate genes that genically interact with \textit{rnt-1}, we performed RNAi screening for genes whose RNAi caused an obvious degenerative phenotype only in the \textit{rnt-1(ok351)} background, but not in the wild-type background (Figure 1B). Among approximately 5000 genes on chromosomes I and III that we screened, we found that seven genes caused an \textit{rnt-1}–dependent synthetic phenotype (Figure 1C, summarized in Table 1).

![Figure 1](image)

**Table 1** Genes that were positively selected with severe morphological defects

| Sequence Name | Gene Name | LG | Description | Phenotype |
|---------------|-----------|----|-------------|-----------|
| C34G6.4      | pgp-2     | I  | ABC transporter family, multidrug resistance family | Rup       |
| F39B2.4      | sur-2     | I  | Mediator complex component, MED23 | Eiv       |
| F39H11.3     | cdk-8     | I  | Cyclin-dependent serine/threonine protein kinase, CDK8 | Eiv       |
| C07G2.3      | cct-5     | III| Cytosolic chaperonin | Rup, Lva  |
| D2045.7      |           | III| Serine/threonine protein kinase/TGFβ-stimulated factor | Emb       |
| H14E04.5     | cic-1     | III| CDK8 kinase-activating protein, Cyclin C | Eiv       |
| W09D10.4     |           | III| Serine/threonine protein phosphatase | Rup       |

Rup, ruptured; Eiv, exploded intestine through vulva; Lva, larval arrest; Emb, embryonic lethality.
embryonic lethality. Among them, cdk-8, cic-1, and sur-2 can be grouped as components of the transcriptional machinery, ppg-2 and cct-5 can be grouped as stress-related genes, and D2045.7 and W09D10.4 can be grouped as components of signal cascade, as categorized by the functional GO terms. cdk-8 and cic-1 are orthologs of the components of the CDK8 mediator complex, CDK8 and CycC, respectively. sur-2 is the ortholog of MED23, which is a member of the mediator complex. The mediator complex is a core regulatory integrator that links DNA binding factors and transcriptional machinery (Casamassimi and Napoli 2007). These results suggest that the function of RNT-1 as a transcription factor is closely related with specific transcriptional machinery components in C. elegans. ppg-2 was cloned as an ortholog of multidrug resistance gene (Lincke et al. 1992) and is required for the formation of gut granules and lysosome-related fat storage (Schroeder et al. 2007). cct-5 is a component of cytosolic chaperonin (Leroux and Candido 1995). It was reported that knock-down of cct-5 might increase the expression of gst-4 through increased oxidative stress evoked by unidentified mechanisms (Kahn et al. 2008). The fact that rnt-1 responds to environmental stress (Lee et al. 2012) led us to speculate that a severe defect in the response to stresses may have induced the synthetic phenotype observed. It would be of interest to dissect the molecular aspects of the synthetic phenotypes caused by the reduction of both cct-5 and rnt-1 genes. In addition, the mechanism of RNT-1 interaction with signaling components such as D2045.7 and W09D10.4 may shed light on the new action mode of RUNX proteins.

The bro-1 gene, located on chromosome I, is a cofactor of RNT-1, and double mutations in rnt-1 and bro-1 are known to cause a similar phenotype as the exploded intestine through the vulva (Kagoshima et al. 2007). However, because bro-1 RNAi clone was not included in the Ahringer library, we have not tested the gene in our screening. Likewise, we may have missed additional genes that interact with rnt-1.
because we did not screen genes on chromosomes other than chromosomes I and III. Screening of more chromosomes may result in the identification of new players in RNT-1 action.

CDK8 mediator complex genetically interacts with rnt-1

RNAi of cdk-8 and cic-1 in the rnt-1 mutant background caused a similar phenotype to each other, that is, the Eiv phenotype (Figure 2A). We examined the possibility that other components of the CDK8 mediator complex such as dpy-22 and let-19 also genetically interact with rnt-1. When we knocked down dpy-22 or let-19 in the rnt-1 mutant background, we obtained the same Eiv phenotype, but not in the wild-type background (Figure 2A). We tested three deletion mutations, rnt-1(ok351), rnt-1(tm388), and rnt-1(tm491), and we observed similar effects (Figure 2A). Interestingly, RNAi of cdk-8 and dpy-22 greatly increased the incidence of Eiv phenotype compared to RNAi of other components. To further clarify the genetic interaction between rnt-1 and cdk-8, we generated and examined the rnt-1(ok351) cdk-8(tm1238) double mutants. The double mutants were maintained only as heterozygous for rnt-1 because of the lethality attributable to protruded intestine through the vulva phenotype. We found that 95.4% of the double mutants, which were confirmed by single-worm PCR, exhibited the Eiv phenotype (n = 22; Figure 2C), whereas rnt-1(ok351) single-mutant animals and cdk-8(tm1238) single-mutant animals showed 0% (n >100) and 5% Eiv phenotype (n = 78), respectively.

An interesting observation was that the Eiv phenotype was such that the intestine, not the egg-laying apparatus, was spilled through the exposed vulva. One explanation for this phenotype could be that the cell cycles in the hypodermis or vulval cells were deregulated by rnt-1 and cdk-8 defects. To test this possibility, we examined the numbers of vulval precursor cells (VPCs) and of hypodermal seam cells. However, we could not identify significant differences (data not shown), suggesting that there might be other mechanisms that are regulated by both rnt-1 and the CDK8 mediator complex near VPCs and hypodermal seam cells. It would be of interest to examine whether RNT-1 and the CDK8-containing mediator complex are involved in the regulation of signal transduction pathways such as TGFβ and WNT pathways, which may be responsible for the differentiation of hypodermal cells.

acs-4 is a putative target gene of RNT-1 and CDK8 mediator complex

To identify genes that are regulated by RNT-1 and the CDK8 mediator complex, we searched for genes that are reported to cause similar phenotype to our exploded intestine through the vulva phenotype. The keywords to extract the candidate genes from the WormBase database are “RNT-1 and the CDK8 mediator complex” and “a similar phenotype to each other, that is, the Eiv phenotype.” Among them, we selected those with the rnt-1 binding site(s) in their promoter regions (Supporting Information, Table S1). We then performed RNAi experiments of these candidate genes to determine whether their RNAi caused the same phenotype as cdk-8 RNAi in rnt-1 mutants. We also measured the amount of transcripts of putative target genes by real-time quantitative PCR to determine whether their transcription was dependent on RNT-1 and CDK8. We found that acs-4 was a gene from the list that showed the Eiv phenotype and was coregulated by rnt-1 and cdk-8 (Figure 3A). The transcript of acs-4 was decreased only when both rnt-1 and cdk-8 were knocked down. We confirmed that the Eiv phenotype was induced by RNAi of acs-4 alone in the wild-type background (Figure 3B and C) at a lower frequency than the cdk-8 phenotype in the rnt-1 mutant background, suggesting that acs-4 is not the only target gene. acs-4 encodes a fatty acyl CoA synthase and its role has been studied in terms of metabolism. Metabolic problems cause a variety of defects in development. Deprivation of fatty acids as a result of mutation in lipid metabolism causes developmental defects such as embryonic lethality and larval arrest in C. elegans, which are triggered by incomplete cytoskeletal components, reduction of function in signaling pathway, and abnormal biophysical property of membrane (Vrablik and Watts 2012). It has been recently reported that fatty-acid biosynthesis affects the polarity of intestine cells in C. elegans (Zhang et al. 2011). The intestine of C. elegans stores lots of fatty acids. It is conceivable that deregulation of acs-4, which is regulated by rnt-1 and CDK8 mediator complex, may have led to
abnormal lipid status of the worms, which has resulted in the Eiv phenotype in general.

In summary, our results suggest that rnt-1 may physically interact with the CDK8 mediator complex in C. elegans. Consistent with our results, physical and genetic interactions between Lozenge, a RUNX gene, and CDK8 mediator complex have recently been reported in flies (Gobert et al. 2010). Taken together, we propose that the action mechanism of RUNX family proteins may be evolutionarily conserved, and that it would be of a great interest to establish the role of CDK8 mediator complex in the function of RUNX proteins in mammals.

ACKNOWLEDGMENTS
We thank J. Ahn for personal communication. This work was supported by the Research Center for Functional Cellomics (2013003868) and by Priority Research Centers Program (2012-048067) funded by the Ministry of Education, Korea.

LITERATURE CITED
Aberg, T., A. Cavender, J. S. Gaikwad, A. L. Bronckers, X. Wang et al., 2004 Phenotypic changes in dentition of Runx2 homozygote-null mutant mice. J. Histochem. Cytochem. 52: 131–139.
Bae, S. C., and Y. H. Lee, 2006 Phosphorylation, acetylation and ubiquitination: the molecular basis of RUNX regulation. Gene 366: 58–66.
Banerjee, C., L. R. McCabe, J. Y. Choi, S. W. Hiebert, J. L. Stein et al., 1997 Run homology domain proteins in osteoblast differentiation: AML3/CBF1 is a major component of a bone-specific complex. J. Cell. Biochem. 66: 1–8.
Bangswor, C., N. Rubins, G. Glusman, Y. Bernstein, V. Negreanu et al., 2001 The RUNX3 gene–sequence, structure and regulated expression. Gene 279: 221–232.
Blyth, K., E. R. Cameron, and J. C. Neil, 2005 The RUNX genes: gain or loss of function in cancer. Nat. Rev. Cancer 5: 376–387.
Braun, T., and A. Woollard, 2009 RUNX factors in development: lessons from invertebrate model systems. Blood Cells Mol. Dis. 43: 43–48.
Brenner, S., 1974 The genetics of Caenorhabditis elegans. Genetics 77: 71–94.
Casamassimi, A., and C. Napoli, 2007 Mediator complexes and eukaryotic transcription regulation: an overview. Biochimie 89: 1439–1446.
Coffman, J. A., 2003 Runx transcription factors and the developmental balance between cell proliferation and differentiation. Cell Biol. Int. 27: 315–324.
Cohen, M. M., Jr., 2009 Perspectives on RUNX genes: an update. Am. J. Med. Genet. A. 149A: 2629–2646.
Fukamachi, H., and K. Ito, 2004 Growth regulation of gastric epithelial cells by Runx3. Oncogene 23: 4330–4335.
Gobert, V., D. Osman, S. Bras, B. Auge, M. Boube et al., 2010 A genome-wide RNA interference screen identifies a differential role of the mediator CDK8 module subunits for GATA/ RUNX-activated transcription in Drosophila. Mol. Cell. Biol. 30: 2837–2848.
Kagoshima, H., K. Shigesada, and Y. Kohara, 2007 RUNX regulates stem cell proliferation and differentiation: insights from studies of C. elegans. J. Cell. Biochem. 100: 1119–1130.
Kahn, N. W., S. L. Rea, S. Moyle, A. Bell, and T. E. Johnson, 2008 Proteasomal dysfunction activates the transcription factor SKN-1 and produces a selective oxidative-stress response in Caenorhabditis elegans. Biochem. J. 409: 205–213.
Lee, J., J. Ahhn, and S. C. Bae, 2004 Homologs of RUNX and CBF beta/PEBP2 beta in C. elegans. Oncogene 23: 4346–4352.
Lee, K., J. Shim, J. Bae, Y. J. Kim, and J. Lee, 2012 Stabilization of RNT-1 protein, runt-related transcription factor (RUNX) protein homolog of Caenorhabditis elegans, by oxidative stress through mitogen-activated protein kinase pathway. J. Biol. Chem. 287: 10444–10452.
Leroux, M. R., and E. P. Candido, 1995 Characterization of four new tcp-1-related cct genes from the nematode Caenorhabditis elegans. DNA Cell Biol. 14: 951–960.
Lincke, C. R., I. The, M. van Groenigen, and P. Borst, 1992 The P-glycoprotein gene family of Caenorhabditis elegans. Cloning and characterization of genomic and complementary DNA sequences. J. Mol. Biol. 228: 701–711.
Michaud, J., F. Wu, M. Osato, G. M. Cottles, M. Yanagida et al., 2002 In vitro analyses of known and novel RUNX1/AML1 mutations in dominant familial platelet disorder with predisposition to acute myelogenous leukemia: implications for mechanisms of pathogenesis. Blood 99: 3546–3572.
Mundlos, S., F. Otto, C. Mundlos, J. B. Mulliken, A. S. Aylsworth et al., 1997 Mutations involving the transcription factor CBFA1 cause cleidocranial dysplasia. Cell 89: 773–779.
Nam, S., Y. H. Jin, Q. L. Li, K. Y. Lee, G. B. Jeong et al., 2002 Expression pattern, regulation, and biological role of runt domain transcription factor, run, in Caenorhabditis elegans. Mol. Cell. Biol. 22: 547–554.
Nimmo, R., A. Antebi, and A. Woollard, 2005 mab-2 encodes RNT-1, a C. elegans Runx homologue essential for controlling cell proliferation in a stem cell-like developmental lineage. Development 132: 5043–5054.
Osato, M., 2004 Point mutations in the RUNX1/AML1 gene: another actor in RUNX leukemia. Oncogene 23: 4284–4296.
Osato, M., N. Asou, E. Abdalla, K. Hoshino, H. Yamasaki et al., 1999 Biallelic and heterozygous point mutations in the runt domain of the AML1/PEBP2alpha gene associated with myeloblastic leukemias. Blood 93: 1817–1824.
Otto, F., A. P. Thornell, T. Crompton, A. Denzel, K. C. Gilmour et al., 1997 Cbfa1, a candidate gene for cleidocranial dysplasia syndrome, is essential for osteoblast differentiation and bone development. Cell 89: 765–771.
Schroeder, L. K., S. Kremer, M. J. Kramer, E. Currie, E. Kwan et al., 2007 Function of the Caenorhabditis elegans ABC transporter PGP-2 in the biogenesis of a lysosome-related fat storage organelle. Mol. Biol. Cell 18: 995–1008.
Shim, J., and J. Lee, 2008 Regulation of rnt-1 expression mediated by the opposing effects of BRO-1 and DBL-1 in the nematode Caenorhabditis elegans. Biochem. Biophys. Res. Commun. 367: 130–136.
Song, W. J., M. G. Sullivan, R. D. Legare, S. Hutchings, X. Tan et al., 1999 Haploinsufficiency of CBFA2 causes familial thrombocytopenia with propensity to develop acute myelogenous leukaemia. Nat. Genet. 23: 166–175.
Thastrup, O., P. J. Cullen, B. K. Drobak, M. R. Hanley, and A. P. Dawson, 1990 Thapsigargin, a tumor promoter, discharges intracellular Ca2+ stores by specific inhibition of the endoplasmic reticulum Ca2+-ATPase. Proc. Natl. Acad. Sci. USA 87: 2466–2470.
Vrablik, T. L., and J. L. Watts, 2012 Emerging roles for specific fatty acids in developmental processes. Genes Dev. 26: 631–637.
Wang, Q., T. Stacy, M. Binder, M. Marin-Padilla, A. H. Sharpe et al., 1996 Disruption of the Cbfa2 gene causes necrosis and hemorrhaging in the central nervous system and blocks definitive hematopoiesis. Proc. Natl. Acad. Sci. USA 93: 3444–3449.
Werner, M. H., K. Shigesada, and Y. Ito, 1999 Runt domains take the lead in hematopoiesis and osteogenesis. Nat. Med. 5: 1356–1357.
Zhang, H., N. Abraham, L. A. Khan, D. H. Hall, J. T. Fleming et al., 2011 Apico basal domain identities of expanding tubular membranes depend on glycosphingolipid biosynthesis. Nat. Cell Biol. 13: 1189–1201.

Communicating editor: D. S. Fay