Absence of Erythropoiesis and Vasculogenesis in Plcg1-deficient Mice*

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Mice nullizygous for Plcg1 cease growing at early to mid-gestation. An examination of carefully preserved wild-type embryos shows clear evidence of erythropoiesis, but erythropoiesis is not evident in Plcg1 nullizygous embryos at the same stage. The analyses of embryonic materials demonstrate that in the absence of Plcg1, erythroid progenitors cannot be detected in the yolk sac or embryo body by three different assays, burst-forming units, colony-forming units, and analysis for the developmental marker Ter119. However, non-erythroid granulocyte/macrophage colonies are produced by Plcg1 null embryos. Further analysis of these embryos demonstrates significantly diminished vasculogenesis in Plcg1 nullizygous embryos based on the lack of expression of the endothelial marker platelet endothelial cell adhesion molecule-1. In addition, Plcg1 nullizygous embryos express a greatly reduced level of vascular endothelial growth factor receptor-2/Flk-1, consistent with significational impairement vasculogenesis and erythropoiesis. Interestingly, these early embryos do express phospholipase C-γ2, however, it is unable to substitute for the absence of phospholipase C-γ1, which can be detected in its tyrosine-phosphorylated state.

Phosphatidylinositol-specific phospholipase C (PLC)1 isozymes catalyze the hydrolysis of phosphatidylinositol 4,5-bisphosphate to produce the second messenger molecules inositol 1,4,5-trisphosphate and diacylglycerol (1). Inositol 1,4,5-trisphosphate provokes the mobilization of intracellular Ca²⁺ to increase the intracellular level of free Ca²⁺, whereas diacylglycerol serves as an endogenous activator of protein kinase C (2).

The family of phosphatidylinositol-PLC isoforms is divided into subtypes based on the presence of functional domains and sequence similarities. These subtypes include four well described PLC-β isoforms, two PLC-γ isoforms, four PLC-δ isoforms (3), and one recently reported PLC-ε isoform (4–6). Of these, only the γ isoforms are known to be activated by receptor and non-receptor tyrosine kinases, whereas the β isoforms are effectors of G protein-coupled receptors. The cellular regulation of the δ isoforms is unknown, whereas the ε isoform is thought to interact with Ras through an uncertain mechanism.

Although catalytic subdomains X and Y are common to all phosphatidylinositol-PLC isoforms, PLC-γ isoforms uniquely contain two SH2 domains and one SH3 domain. The two SH2 domains facilitate an association of PLC-γ isoforms with and phosphorylation by receptor tyrosine kinases, whereas the function of the SH3 domain is not yet defined (3). The mechanism by which PLC-γ1 promotes mitogenic responses to growth factors is not understood (3), although it has been demonstrated recently that PLC-γ1 is necessary to promote the induction of immediate early genes by growth factors (7–9).

Several Plc genes including Plcg1 (10) and Plcg2 (11) have been analyzed by targeted disruption in mice. Only the homozgyous disruption of Plcg1 or Plcg2 results in embryonic lethality. Plcβ3 produces lethality at E2.5 prior to implantation (12), whereas Plcg1 results in lethality soon after E9.0 apparently because of a generalized growth failure. Heterozygous Plcg1 embryos are not detectable different from wild-type embryos. Interestingly, mice genetically deficient in Plcg2 have a mild phenotype consisting of depressed B cell numbers and impaired mast cell function (11). The Plc-γ1 and Plc-γ2 isoforms differ mainly in their patterns of expression. The γ1 isoform is ubiquitously expressed in most all tissues (13), whereas the γ2 isoform is selectively expressed in the spleen and thymus (14). A Drosophila PLC-γ gene has been reported previously (15) and exhibits a loss-of-function phenotype called “small wing,” which includes diminished wing size with altered blood vessel morphogenesis.

As described in this manuscript, we have determined whether development of the hematopoietic and vascular systems are impaired in mice that are deficient in Plcg1 as a possible reason for the observed embryonic lethality. We have also examined the expression of the PLC-γ1 and PLC-γ2 isoforms and their tyrosine phosphorylation status in these early embryos.

EXPERIMENTAL PROCEDURES

Embryos and Genotyping—Targeting of the Plcg1 gene described previously (10) results in the deletion of exons encoding the X catalytic subdomain in addition to both SH2 domains. Plcg1 heterozygotes were interbred in a 129SV × C57 genetic background. Plcg1 heterozygous female mice were caged with a Plcg1 heterozygous male mouse overnight. When the females were examined early next morning, the appearance of the vaginal plug was designated as day 0.5 of gestation (16). At the indicated times of gestation, pregnant female mice were anes-
thetized and sacrificed. The embryos were removed, photographed, and dissected under a dissecting microscope to obtain the yolk sac and embryo body. A small piece of yolk sac was then removed for genotyping as described previously (10).

**Colony-forming Unit-Erythroid (CFU-E) and Burst-forming Unit-Erythroid (BFU-E) Assays**—To produce embryonic single cells, the yolk sac or embryo body was digested for 3 h at 37 °C with intermittent agitation with collagenase (Sigma) at a final concentration of 0.1% in phosphate-buffered saline (PBS) without calcium and magnesium but with 20% fetal bovine serum (17). At the end of the incubation, the digestion mixture was allowed to settle for 5 min before the supernatant-containing disaggregated embryonic single cells was harvested. Digestion mixture was allowed to settle for 5 min before the supernatant-containing disaggregated embryonic single cells was harvested. The erythroid colony formation assays were performed as described previously (18, 19). Disaggeregated embryonic cells were seeded in a six-well plate with culture medium containing α-minimal essential medium, 0.8% (v/v) methylcellulose, 30%, (v/v) fetal bovine serum, and 100 μM 2-mercaptopethanol. For the CFU-E assay, 2 units/ml of erythropoietin (EPO) was added, and for the BFU-E assay, 10 ng/ml of interleukin-3 and 100 ng/ml of stem cell factor were added in addition to 2 units/ml of EPO. All cytokines were purchased from R&D Systems, Inc. CFU-E colonies were counted at 3 days of culture, whereas BFU-E and granulocyte/macrophage colonies were counted at 7–10 days of culture. Colony-type determination was based on size, morphology, and color as described previously (18, 19).

The data presented on the CFU-E assay were obtained from one litter at E7.5 and four litters at E8.5 that includes 16+/+ embryos, 42−/−embryos, and 13+/−embryos. The data presented in the BFU-E assay are the average of two litters at E8.0 and E8.5 representing 9+/+ embryos, 13−/+embryos, and 5−/−embryos.

**Immunohistological Stains**—Whole mount immunostaining was performed essentially as described previously (20). Embryos were fixed for 1 h at 4 °C in 4% paraformaldehyde, washed three times with PBS, dehydrated in a graded series of methanol/PBS, and then stored in 100% methanol at −20 °C until use. Subsequently, embryos were bleached in 6% hydrogen peroxide in methanol for 1 h and then rehydrated in a reverse series of methanol dilutions. The embryos were then blocked in antibody dilution buffer (3% skim milk, 1% Triton X-100 in PBS). The embryos were stained with mouse PECAM-1 monoclonal antibody (PharMingen) or Fk-1 monoclonal antibody (PharMingen) according to the instructions by the manufacturer. Multiple embryos (at least three) of each genotype were stained, and a representative example was presented.

For immunohistofluorescent Ter119 staining, embryos were fixed in 4% paraformaldehyde for 1 h, washed three times with PBS, and then embedded. Cryostat sections were cut at 10 μm, dried, and stained in 100% methanol at −20 °C until use. Subsequently, embryos were bleached in 6% hydrogen peroxide in methanol for 1 h and then rehydrated in a reverse series of methanol dilutions. The embryos were then blocked in antibody dilution buffer (3% skim milk, 1% Triton X-100 in PBS). The embryos were stained with mouse PECAM-1 monoclonal antibody (PharMingen) or Fk-1 monoclonal antibody (PharMingen) according to the instructions by the manufacturer. Multiple embryos (at least three) of each genotype were stained, and a representative example was presented.

**Reverse Transcription-Polymerase Chain Reaction Analysis of Plcg1 mRNA**—Individual yolk sacs or embryo bodies were transferred to an Eppendorf tube containing 50 μl of ice-cold PBS in diethylypyrocarbonate-treated H2O. The PBS solution was then removed from the tube, and 50 μl of lysis buffer (2% Triton X-100, 2 units/μl of RNase inhibitor, 5 mM dithiothreitol) was added and incubated on ice for 60 min. Lysed tissue mixtures were stored at −80 °C.

For RT-PCR, the lysed tissue mixture was thawed and centrifuged 16,000 × g for 10 min. 5 μl of the supernatant was then transferred to a RT-PCR tube and was reverse transcribed to first-strand cDNA using a SuperScript II kit (GIBCOBRL) according to the instructions by the manufacturer. Nested PCR was carried out under standard conditions (22). The partial mouse Plcg-2 DNA sequence was kindly provided by James N. Ihle (St. Jude Children’s Research Hospital, Memphis, TN). A pair of primers was sense primer 5’-GGAGCTGAGAACCACATCTTGCC-3’ and antisense primer 5’-GACCTTTGTCGAGACTCC-3’. These primers were predicted to amplify a cDNA 305 bp in length. The second pair of primers was sense primer 5’-AAGGCTTTCACAGGACAAGCTGG-3’ and antisense primer 5’-TTGGAAGTCTCTTGCAAGGTAG-3’. These primers were predicted to amplify a cDNA 201 bp in length.

**RESULTS**

**Morphology of Plcg1Nullizygous Embryos**—As previously reported, Plcg1−/− embryos undergo severe growth retardation beginning approximately at embryonic day 9.0 (10), although the heart remains beating at E9.5 but not at E10.5. Hence, embryonic lethality occurs between E9.5 and 10.5. Close inspection of carefully preserved E9.0 embryos reveals that blood islands are readily detectable in the wild-type embryo yolk sac but are not detectable in the yolk sac of Plcg1 null embryos (Fig. 1). These observations suggest that the development of critical elements of the embryonic blood formation is impaired in the absence of Plcg-1.

**Erythropoiesis in the Absence of Plcg1**—To determine whether the absence of Plcg-1 during mouse embryogenesis interferes with the formation of mature erythroid cells, the erythropoietin-dependent CFU-E assay was employed. Cells from collagenase-digested yolk sacs or embryo bodies of Plcg1+/+, Plcg1−/+ , and Plcg1−/− embryos (approximately E8.0) were incubated with erythropoietin for 3 days, and the number of erythroid colonies produced was determined. As shown in Fig. 2A, erythroid colonies were readily produced with cells obtained from the yolk sacs of Plcg1 wild-type and heterozygous embryos, but no detectable colonies were produced with yolk sac cells from Plcg1 nullizygous embryos. In this assay, significant numbers of erythroyocyte colonies were not detectable in assays that used only the embryo body.

To detect an earlier erythroid progenitor and to assess the presence of progenitors for a non-erythrocyte lineage, the BFU-E assays were conducted. Whole embryos, i.e. embryo body plus yolk sac (E8.0–E8.5) were disaggregated, and the cells incubated for at least 7 days with multiple cytokines (EPO, interleukin-3, and stem cell factor). Subsequently, the numbers of erythroid colonies, granulocyte/macrophage colonies, and mixed colonies of both types were counted (Fig. 2B). The results of this assay show that the BFU-E assays were performed in the absence of progenitors for non-erythrocyte cell types, and the number of erythroid colonies was significantly decreased (~80%) with cells obtained from Plcg1 nullizygous embryos but not entirely absent as in the CFU-E assay. This suggests that erythroid progenitors in reduced numbers are present in Plcg1 nullizygous embryos. Also, the formation of granulocyte/macrophage colonies in the BFU-E assay was not significantly impaired by the Plcg1−/− genotype.
The data obtained from the CFU-E and BFU-E assays in vitro indicate the near absence of erythroid progenitors in Plcg1−/− embryos. To establish this in the embryos more directly, the presence of the erythroid progenitor marker Ter119 (23) was examined by immunofluorescence staining of yolk sac sections from E9.5 Plcg1+/+ and Plcg1−/− embryos. As shown in Fig. 3, Ter119 was readily detectable on blood islands within the yolk sac of wild-type embryos but was undetectable in the yolk sac of Plcg1 nullizygous embryos, indicative of significantly diminished primitive erythropoiesis. The results of Ter119 staining in Plcg1 wild-type and nullizygous embryo body were similar to that observed in the yolk sac (data not shown).

Vasculogenesis in Plcg1 Nullizygous Embryos—The morphologic absence of blood island in Plcg1−/− embryos (Fig. 1) could be because of a lack of erythropoiesis and/or the failure of the embryos to develop endothelial cells or to undergo endothelial morphogenesis. Therefore, wild-type and Plcg1-deficient embryos were examined by immunohistochemistry for the presence of an endothelial marker, the adhesion molecule PECAM-1. As shown in Fig. 4, PECAM-1 was readily detected in vessels of the yolk sac, allantois, and embryo body from wild-type embryos at E8.5. However, no PECAM-1 staining was detectable in Plcg1 nullizygous embryos.

Endothelial and hematopoietic development is dependent on the expression of vascular endothelial growth factor system (24). Therefore, we have determined whether the VEGFR-2/flk-1 molecule is expressed in Plcg1-deficient embryos. As shown in Fig. 5, VEGFR-2/flk-1 reactivity was detected in blood vessels of the yolk sac and embryo body of Plcg1+/+ embryos, but no staining was detected in the yolk sac of Plcg1−/− embryos, and only very weak staining was detected in the embryo body. However, VEGFR-2/flk-1 staining was present in the allantois of embryos regardless of the Plcg1 genotype, indicating that some cells expressing this receptor are present in the mutant embryos. Quinn et al. (25) have reported that the non-endothelial cells in the stroma of the umbilical cord express very high levels of flk-1 at E12.5. Also, Millauer et al. (26) have described Flk-1 expression in the mesenchyme of the allantois at E8.5. The Flk-1 expression observed in this region of Plcg1 null embryos most probably represents non-endothelial expression that is independent of Plcg1.

**Fig. 1.** Morphology of Plcg1 wild-type and nullizygous embryo. Embryos were removed at E9.0 and genotyped.

**Fig. 2.** Influence of Plcg1 genotype on CFU-E and BFU-E assays. A, CFU-E assays were performed using yolk sacs or embryo bodies isolated from Plcg1 wild-type, heterozygous, and nullizygous embryos. B, BFU-E assays were performed with whole embryos of each Plcg1 genotype. In each assay, cells from collagenase-digested embryonic tissues were incubated in medium containing EPO (2 units/ml) for the CFU-E assay or EPO (2 units/ml), interleukin-3 (10 ng/ml), and stem cell factor (100 ng/ml) for the BFU-E assay. CFU-E colonies were counted at ~3 days of culture, whereas BFU-E colonies were counted after 7–10 days of culture.
Expression of PLC-γ Isozymes—The results noted above imply that PLC-γ1 is expressed in both yolk sac and embryo body by E9.5. This is demonstrated in Fig. 6 by whole mount β-galactosidase staining of a Plcg1 heterozygous embryo (E9.5) that is derived from a TV-II-targeted disruption of the Plcg1 allele, which results in the expression of a fusion protein containing the amino terminus of PLC-γ1 and β-galactosidase expressed from the Plcg1 promoter (10). As shown in Fig. 6, β-galactosidase staining of the yolk sac demonstrates widespread expression of the enzyme. Abundant expression is also present in the embryo body, particularly in the first brachial arch, midline dorsal aorta, limbs, and allantois as noted previously (10). Also, enzyme expression in the vasculature is readily apparent. In Plcg1−/− embryos, β-galactosidase staining was present in the yolk sac and embryo body, but no staining of the vasculature was apparent (data not shown).

For more detailed analysis of PLC-γ1 distribution in embryos, the same Plcg1 heterozygous embryo (Fig. 6, A and B)
was sectioned and stained with eosin. As shown in Fig. 6C, β-galactosidase is expressed widely in almost all embryonic tissues, particularly in the gut, endothelium of the dorsal aorta, amnion, plus the endoderm and mesothelium of the yolk sac.

The expression of PLC-γ1 and PLC-γ2 was also examined by Western blotting of E9.5 embryos. As shown in Fig. 7A, PLC-γ2 was detected in both Pieg1+/+ and Pieg1−/− whole embryos, whereas as expected, PLC-γ1 was only present in the wild-type embryos. Subsequently, wild-type yolk sacs and embryo bodies were separately analyzed for the expression of PLC-γ1 and PLC-γ2 (Fig. 7A). Both γ1 and γ2 isoforms are present in the yolk sacs and embryo bodies of these wild-type embryos.

Because both PLC-γ1 isozymes are expressed in E9.5 embryos and yolk sacs, each isozyme was tested for the presence of phosphotyrosine by immunoprecipitation with the appropriate isozyme antibody followed by Western blotting with anti-phosphotyrosine. The presence of phosphotyrosine would indicate that the isozyme is biochemically activated in embryonic tissue by an unknown tyrosine kinase. As shown in Fig. 7B, lanes 1 and 2, tyrosine-phosphorylated PLC-γ1 was detectable in both the yolk sac and embryo body. As a gel marker for this analysis (lanes 3 and 4), we also immunooanalyzed the tyrosine phosphorylation of PLC-γ1 in control and epidermal growth factor-treated mouse embryo fibroblasts. When the same embryonic material was analyzed for PLC-γ2, no tyrosine phosphorylation was detected (data not shown), although the data in Fig. 7A show that a significant level of PLC-γ2 is present in these tissues. These results indicate that PLC-γ1, but not PLC-γ2, is activated in the yolk sac and embryo body at this point in gestation.

Finally, RT-PCR was employed to assess the expression of PLC-γ2 mRNA in both Pieg1 wild-type and nullizygous embryos. RT-PCR instead of Western blotting was employed to assess γ2 expression because of the small size of the Pieg1−/− embryos. The results shown in Fig. 8 indicate that regardless of the Pieg1 genotype, PLC-γ2 mRNA is expressed in the embryo body. However, the expression of this mRNA in the yolk sac was only detected in Pieg1+/+ embryos. Hence, the expression of PLC-γ2 mRNA in the yolk sac is dependent on the presence of PLC-γ1.

DISCUSSION

The circulatory system is the first and most essential organ system to begin functioning during embryonic development. In various ways and at slightly different gestational times (E7.0–E11.0), the deletion of a number of signal transduction path-
knockouts, e.g. VEGFR-2/Flk-1 (28, 29), not only are the endothelial and erythroid lineages absent, non-erythroid hematopoietic lineages also are not present. In the case of Plcg1 nullizygous embryos, non-erythroid granulocyte/macrophage colonies are detected in the BFU-E assay in amounts comparable to Plcg1 wild-type embryos. Also, in the BFU-E assay that detects an earlier erythroid progenitor than the CFU-E assay (18, 19), a small number of erythroid colonies are detected in the BFU-E assay in amounts comparable to Plcg1 wild-type embryos. In Plcg1 nullizygous and wild-type whole embryos at E9.5 or wild-type yolk sacs and embryo bodies at E9.5 were Western blotted with antibody to PLC-γ1 or PLC-γ2 as indicated. B, aliquots (1 mg of protein) of lysates from wild-type yolk sacs or embryo bodies (E9.5) were precipitated with PLC-γ1 antibody and blotted with anti-phosphotyrosine (upper panel, lanes 1 and 2). The blots were then stripped and reprobed with PLC-γ1 antibody (lower panel, lanes 1 and 2). As a gel marker (lanes 3 and 4), quiescent mouse embryo fibroblasts were stimulated for 5 min without or with epidermal growth factor (25 ng/ml), and lysate aliquots (500 µg protein) were immunoprecipitated with anti-PLC-γ1, blotted with anti-phosphotyrosine, stripped, and rebotted with anti-PLC-γ1.

VEGF receptors are known to interact with PLC-γ1 (31, 32), and the loss of these receptors particularly VEGFR-1/Flt-1 (33) or VEGFR-2/Flk-1 (28, 29) produces an embryonic lethal phenotype between E8.5 and E9.5, close to that presented for Plcg1 nullizygous embryos. Hence, it is possible that PLC-γ1 is an essential VEGF substrate in the development of early endothelial and erythroid progenitors but is not a critical substrate in VEGF-dependent formation of non-erythroid lineages. A comparison of E9.5 embryos null for Plcg1 (10) or VEGFR-2/Flk-1 (28) or VEGFR-1/Flt-1 (33) suggests that Plcg1 deficiency produces a more deleterious phenotype than the loss of either receptor. The phenotype of mice doubly deficient in VEGF receptors has not been described. Hence, at this point in development, PLC-γ1 may be a required substrate for both VEGF receptors and/or another receptor kinase(s). However, because the loss of the VEGF-1/Flt-1 receptor kinase activity does not impair mouse development (34), the latter possibility seems more probable.

In Plcg1 nullizygous embryos, there is an increased susceptibility to apoptosis induced by either anokiasis or oxidative stress (35). Also, the overexpression of PLC-γ1 in P12 cells is reported to attenuate ultraviolet C-induced apoptosis (36). Hence, it seems possible that in early endothelial and erythroid progenitors, PLC-γ1 may have a role in signaling pathways for cell survival and/or cell proliferation.

It was perhaps surprising that the loss of the gene for PLC-γ2 produced a relatively mild phenotype (11) given the selective expression of this isoform in the spleen and thymus. Our data show that both PLC-γ isoforms are present at E9.5 in the yolk sac where erythropoiesis and vasculogenesis are initiated as well as the embryo body. Hence, it might be expected that the two isoforms would be functional-redundant, but obviously they are not. This observation could be explained if two isoforms interact selectively with different activated receptors tyrosine kinases based on the specificity differences of their respective SH2 domains. For example, reported studies (37) indicate that PLC-γ1 does not associate with the colony-stimulating factor-1 receptor, but that PLC-γ2 does associate with this receptor (38). It is also possible that in these early embryos, PLC-γ1 and PLC-γ2 are in fact expressed in different cell types within the yolk sac and embryo body.

Interestingly, our data show that PLC-γ2 mRNA is not de-
tected in the yolk sac of Plcg1 nullizygous embryos, whereas it is found in the embryo body the same embryos and in both the yolk sac and embryo body of Plcg1 wild-type embryos. This finding could be interpreted to indicate that within the yolk sac, PLC-γ1 positive cells, which do not express PLC-γ2, give rise to cells that express both isoforms. Hence, the loss of Plcg1 function in some selected lineages may result in the absence of both isoforms. Such a scenario could also explain why the loss of Plcg2 dose not provoke a more severe phenotype.

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Note Added in Proof—Following review of this manuscript, an analysis of Plcg1−/− chimeric mice has demonstrated the necessity of Plcg1 for hematopoiesis and renal development (39).

REFERENCES

1. Berridge, M. J., and Irvine, R. F. (1984) Nature 312, 315–321
2. Nishizuka, Y. (1984) Nature 306, 695–697
3. Carpenter, G., and Ji, Q.-S. (1999) Exp. Cell Res. 253, 15–24
4. Kelley, G. G., Reks, S. E., Ondrako, J. M., and Smrcka, A. V. (2001) EMBO J. 20, 743–754
5. Song, C., Hu, C. D., Masago, M., Kariya, K., Yamawaki-Kataoka, Y., Shihata, H., Mu, D., Satoh, T., and Kataoka, T. (2001) J. Biol. Chem. 276, 2752–2757
6. Lopez, I. I., Mak, E. C., Ding, J., Hamm, H. E., and Lomasney, J. W. (2001) J. Biol. Chem. 276, 2768–2772
7. Wang, Z., Guo, S., Zhang, L., and Moron, M. F. (1998) Mol. Cell. Biol. 18, 590–597
8. Boche, S., McGlade, J., Jones, M., Gish, G. D., Pawson, T., and Courtneidge, S. A. (1996) EMBO J. 15, 4940–4948
9. Liao, H.-J., Ji, Q.-S., and Carpenter, G. (2001) J. Biol. Chem. 276, 8627–8630
10. Ji, Q.-S., Winnier, G. E., Niswender, K. D., Horstman, D., Wisdom, R., Magnuson, M. A., and Carpenter, G. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 2999–3003
11. Wang, D., Feng, J., Wen, R., Marine, J. C., Sangster, M. Y., Pargan, E., Hoffmeyer, A., Jackson, C. W., Cleveland, J. L., Murray, P. J., and Ihle, J. N. (2000) Immunity 13, 25–35
12. Wang, S., Gehrke-Medhin, S., Betsholtz, C., Stalberg, P., Zhou, Y., Larsson, C., Weber, G., Feinstein, R., Oberk, G., Golb, A., and Skogseid, B. (1998) PFESS Lett. 441, 261–265
13. Rhee, S. G., Kim, H., Suh, P. G., and Choi, W. C. (1991) Biochem. Soc. Trans. 19, 337–341
14. Homma, Y., Takenawa, T., Emori, Y., Sorimachi, H., and Suzuki, K. (1989) Biochem. Biophys. Res. Commun. 164, 406–412
15. Thackray, J. R., Gaines, P. C., Ebert, P., and Carlson, J. R. (1998) Development 125, 5033–5042
16. Hogan, R., Beddington, R., Costantini, P., and Lacy, E. (1994) Manipulating the Mouse Embryo: A Laboratory Manual, 2nd Ed, pp. 24–26. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
17. Wong, P. M. C., Chung, S.-W., Reichead, S. M., and Chui, D. H. K. (1986) Blood 67, 716–721
18. Gregory, C. J., and Eaves, A. C. (1977) Blood 855–864
19. Gregory, C. J., and Eaves, A. C. (1978) Blood 51, 527–537
20. Kume, T., Jiang, H., Topczewska, J. M., and Hogan, B. L. (2001) Genes Dev. 15, 2470–2482
21. Arteaga, C. L., Johnson, M. D., Todderud, G., Coffey, R. J., Carpenter, G., and Page, D. L. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 10435–10439
22. Ausubel, F. M., Brent, R., Kingston, R. E., Seidman, J. G., Smith, J. A., and Struhl, K. (1999) Current Protocols in Molecular Biology, John Wiley & Sons, Inc., New York
23. Kina, T., Ikuta, K., Takayama, E., Wada, K., Majumdar, A. S., Weissman, I. L., and Kataoka, T. (2001) Br. J. Haematol. 110, 280–287
24. Daniel, T. O., and Abrahamsson, D. (2000) Annu. Rev. Physiol. 62, 649–671
25. Quinn, T. P., Peters, K. G., De Vries, C., Ferrara, N., and Williams, L. T. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 7533–7537
26. Millard, B., Wizigmann-Voss, S., Schnurch, H., Martinez, R., Moller, N. P., Risau, W., and Ulrich, A. (1993) Cell 72, 839–846
27. Keller, G., Lacaund, G., and Robertson, S. (1999) Exp. Hematol. 27, 777–787
28. Shalaby, F., Rossant, J., Yamaguchi, T. P., Gertsenstein, M., Wu, X. F., Breitman, M. L., and Schub, A. C. (1995) Nature 376, 62–66
29. Shalaby, F., Ho, J., Stanford, W. L., Fischer, K. D., Schuh, A. C., Schwartz, L., Bernstein, A., and Rossant, J. (1997) Cell 89, 981–990
30. Kinder, S. J., Tsang, T. E., Quinlan, G. A., Hadjantonakis, A. K., Nagy, A., and Tam, P. P. (1999) Development 126, 4691–4701
31. Sawano, A., Takahashi, T., Yamaguchi, S., and Shibuya, M. (1997) Biochem. Biophys. Res. Commun. 238, 487–491
32. Takahashi, T., and Shibuya, M. (1997) Oncogene 14, 2079–2089
33. Feng, G. H., Rossant, J., Gertsenstein, M., and Breitman, M. L. (1995) Nature 376, 66–70
34. Hirata, S., Minowa, O., Kunu, J., Noda, T., and Shibuya, M. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 9349–9354
35. Wang, X., McCullough, K. D., Wang, X.-J., Carpenter, G., and Holbrook, N. J. (2001) J. Biol. Chem. 276, 28364–28371
36. Lee, Y. H., Kim, S., Kim, J., Young Kim, K., Kim, M. J., Ryu, S. H., and Suh, P. (1999) Biochem. Biophys. Acta 1440, 235–243
37. Downing, J. R., Margolis, B. L., Zilberstein, A., Ashmun, R. A., Ullrich, A., Sherr, C. J., and Schlessinger, J. (1989) EMBO J. 8, 3345–3350
38. Bourette, R. P., Myles, G. M., Choi, J. L., and Risau, W. (1993) EMBO J. 12, 5880–5883
39. Shirane, M., Sawa, H., Kobayashi, Y., Nakano, T., Kitajima, K., Shinaki, Y., Nagashima, K., and Negishi, J. (2001) Development 128, 5173–5180
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