Using degenerate polymerase chain reaction, we isolated a cDNA encoding a novel 495-amino acid protein from human and mouse adult heart cDNAs and have designated it angiopoietin-related protein-2 (ARP2). The NH2-terminal and COOH-terminal portions of ARP2 contain the characteristic coiled-coil domain and fibrinogen-like domain that are conserved in angiopoietins. ARP2 has two consensus glycosylation sites and a highly hydrophobic region at the NH2 terminus that is typical of a secretory signal sequence. Recombinant ARP2 expressed in COS cells is secreted and glycosylated. In human adult tissues, ARP2 mRNA is most abundant in heart, small intestine, spleen, and stomach. In rat embryos, ARP2 mRNA is most abundant in the blood vessels and skeletal muscles. Endothelial and vascular smooth muscle cells also contain ARP2 mRNA. Recombinant ARP2 protein induces sprouting in vascular endothelial cells but does not bind to the Tie1 or Tie2 receptor. These results suggest that ARP2 may exert a function on endothelial cells through autocrine or paracrine action.

The recent discovery of angiopoietin-1 (Ang1) and angiopoietin-2 (Ang2) has provided insight into the molecular and cellular mechanisms of blood vessel formation (1, 2). Ang1 and Ang2 share about 60% amino acid identity and bind with similar affinity to the endothelial cell tyrosine kinase receptor, Tie2 (1, 2). In vivo analysis by targeted gene inactivation reveals that Ang1 recruits and sustains periendothelial support cells (3), whereas Ang2 disrupts blood vessel formation in the developing embryo by antagonizing the effects of Ang1 on Tie2 (2). Thus, Ang2 is a naturally occurring antagonist of Ang1 that competes for binding to Tie2 and blocks Ang1-induced Tie2 autophosphorylation (2).

Ang1 and Ang2 have characteristic protein structures that contain a coiled-coil domain in the NH2-terminal portion and a fibrinogen-like domain in the COOH-terminal portion (1, 2).

Using homology-based PCR, we previously isolated a cDNA encoding a novel angiopoietin-related protein and designated it angiopoietin-3 (Ang3) (4). However, Valenzuela et al. (5) recently isolated a novel subfamily of angiopoietins and named them mouse angiopoietin-3 (Ang3) and human angiopoietin-4 (Ang4); the two are probably interspecies orthologs. Based on amino acid similarity, their Ang3 and Ang4 are closer to Ang1 and Ang2 than our Ang3. Importantly, their Ang3 and Ang4 bound to the Tie2 receptor but not to the Tie1 receptor. Therefore, we rename our previously isolated Ang3 as angiopoietin-related protein-1 (ARP1).

In this study, we isolated a cDNA encoding another novel angiopoietin-related protein and have designated it angiopoietin-related protein-2 (ARP2) because the amino acid sequence of ARP2 is closest to ARP1. Our results indicate that ARP1 and ARP2 share 59% amino acid identity. Notably, ARP2 was preferentially expressed in the blood vessels and skeletal muscles of developing rat embryos. ARP2 is a glycosylated secretory protein that induces sprouting in endothelial cells, probably through an autocrine or paracrine activity.

EXPERIMENTAL PROCEDURES

Isolation of Human and Mouse ARP2—Partial cDNAs of human and mouse ARP2 were amplified using human and mouse adult heart cDNAs as PCR templates. PCR was performed for 30 cycles at an annealing temperature of 52 °C using sense and antisense degenerate primers representing all possible codons for the following peptides of human Ang1 and Ang2: GYEWLG (Ang1, amino acids 353–358; Ang2, amino acids 353–358) and SNLNGM (Ang1, amino acids 455–460; Ang2, amino acids 453–458) (1, 2). A DNA band of expected size (~300 base pairs) was amplified. The amplified DNA was sequenced by cycle sequencing using the AmpliCycle sequencing kit (Perkin-Elmer Corp.). The novel amplified DNA was cloned into the pCR-Blunt vector (Invitrogen). To clone the remaining coding region, human and mouse adult heart cDNAs were used for rapid amplification of cDNA ends (CLONTECH).

Cell Culture—Human umbilical vein endothelial cells (HUVECs) and porcine pulmonary arterial endothelial cells (PAAECs) were prepared from human umbilical cords and porcine pulmonary arteries by collagenase digestion. Human umbilical vascular smooth muscle cells (HUUVSMCs) were explanted from human umbilical arteries. The endothelial or muscle origin of the cultures was confirmed by the presence of factor VIII or smooth muscle actin detected by immunofluorescence. HUVECs and PAAECs were maintained in M-199 medium supplemented with 20% (v/v) fetal bovine serum. HUUVSMCs and COS-7 cells

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank✿(xi) EBI Data Bank with accession number(s) AF125175 and AF125176.

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†The abbreviations used are: PCR, polymerase chain reaction; HUVEC, human umbilical vein endothelial cell; PAAEC, porcine pulmonary arterial endothelial cell; HUUVSMC, human umbilical vascular smooth muscle cell; E, embryonic day; CMV, cytomegalovirus; PAGE, polyacrylamide gel electrophoresis; VEGF, vascular endothelial growth factor; CHAPS, 3-[3-cholamidopropyl]-dimethylammonio)-1-propane-sulfonate; MC, microcarrier.

Molecular Cloning, Expression, and Characterization of Angiopoietin-related Protein

ANGIPOIETIN-RELATED PROTEIN INDUCES ENDOTHELIAL CELL SPROUTING*

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were maintained in Dulbecco's modified Eagle's medium with 10% (v/v) fetal bovine serum at 37 °C in a 5% CO₂ atmosphere. The primary cultured cells used in this study were between passages 2 and 4.

**Northern Blot Analysis**—A 35S-labeled human ARP2 cDNA probe (nucleotides 1–483) and a 32P-labeled mouse ARP2 cDNA probe (nucleotides 1–483) were labeled by random prime labeling (7) and hybridized to a Northern blot (Clontech) according to the manufacturer's instructions. RNA extraction and Northern blot analysis were performed as described previously (6).

In *Situ* Hybridization in Rat Embryo—In *situ* hybridization was performed essentially as described previously (7). In brief, an ARP2 complementary DNA probe (nucleotides 1–483) cRNA probe was transcribed using the Riboprobe (Promega) with [α-35S]UTP (New England Nuclear). Sections were hybridized overnight with RNase A, washed sequentially in SSC, briefly rinsed in a graded series of ethanol, and dried. Slides were placed in x-ray cassettes, exposed to a-Max film (Amersham Pharmacia Biotech) for 5 days, and developed.

**Expression, Purification, and Detection of Recombinant Proteins—**Human, mouse, Ang1, or VEGF165 cDNA was inserted into the CMV promoter-driven mammalian cell expression vector, pcDNA3.1/Myc-His (Invitrogen). This vector contains a 63-base pair c-Myc tag (EQKISEEDL) and a His₆ tag (HHHHHHH) as open reading frames at the 3'-terminus of the coding region. Each gene construct was transfected into COS-7 cells using LipofectAMINE Plus (Life Technologies, Inc.) and incubated at 37.5 °C for 72 h in Dulbecco's modified Eagle's medium with 2% fetal bovine serum under 5% CO₂. The culture supernatant of the COS-7 cells was transferred to a Ni-NTA agarose column (Qiagen) to purify the recombinant proteins. All buffers contained 0.05% 3-[3-cholamidopropyl]-dimethylammonio]-1-propanesulfonic acid (CHAPS) during protein purification and reconstitution. The binding protein was eluted with pH 8.0 buffer containing 250 mM imidazole. The eluates were dialyzed extensively against 0.05 M Tris-HCl, pH 7.5, 150 mM NaCl (TBS buffer) and were concentrated using Centricon 10 (Amicon) at 4 °C. The salts were removed using a desalting column, and the proteins were reconstituted with TBS buffer. Approximately 10 μg of each recombinant protein was obtained. The purity of this protein was determined by silver staining after SDS-PAGE. The proteins were electrophoresed into nitrocellulose membranes and visualized using Western blot analysis (6). The nitrocellulose membranes were blocked by incubation in blocking buffer, incubated with anti-Myc or anti-His antibody, washed, and incubated with horseradish peroxidase-conjugated secondary antibody, and signals were visualized by chemiluminescent detection according to the manufacturer's protocol (Amersham Pharmacia Biotech). The glycosylation status of the recombinant ARP2 and Ang1 were determined with PNGase-F treatment according to the manufacturer's protocol (New England Biolab).

**Mitogenic and Sprouting Assay for ARP2 in Endothelial Cells—**Mitogenic assays in endothelial cells were performed as described previously (4). HUVECs between second and third passages were plated onto gelatinized 24-well plates (2 × 10⁴ cells/well) in M-199 medium supplemented with 5% (v/v) fetal bovine serum and incubated for 24 h. Purified recombinant proteins were added to the growth medium, and cells were stimulated for 24 h. [3H]Thymidine (Amersham Pharmacia Biotech; 1 μCi/well) was added to the cells, and stimulation was continued for another 24 h. Cells were washed with PBS and were lysed with 0.5 N sodium hydroxide. The incorporated radioactivity was measured by liquid scintillation counting. Each experiment was performed in duplicate. The spraying assay in PPAECs was performed as described previously (8–10). Briefly, PPAECs were grown to confluence on microcarrier (MC) beads (diameter, 175 μm; Sigma) and placed in a 2.5 mg/ml fibrinogen gel (Sigma) containing recombinant VEGF₁₆₅ (10 ng/ml), Ang1 (200 ng/ml), ARP1 (200 ng/ml), or ARP2 (200 ng/ml) and 200 units/ml Trasylol (Bayer). Fibrin gels were incubated in M-199 with a daily supplement of the same amount of recombinant proteins, heat-inactivated fetal bovine serum, and 200 units/ml Trasylol. After 3 days, the extent of sprouting was determined using a phase-contrast inverted microscope (Zeiss).

**In Vitro Binding Assay between ARP2 Protein and Extracellular Domain of Tie1 or Tie2 Receptor—**The secreted extracellular domains of the Tie1 and Tie2 receptors were produced using pFLAG CMV-1 vector (Eastman Kodak Co.), which has a preprotrypsin leader sequence for secretion of the fusion protein. The extracellular domains of human Tie1 (amino acids 28–752) and Tie2 (amino acids 27–729) were cloned into pFLAG CMV-1 in an open reading frame. These constructs were transfected into COS-7 cells, and the culture supernatants were collected for 48 h for the binding assay. These fusion proteins were designed to be purified using Ni-NTA agarose affinity gel (Kodak) was used for holding pFLAG-eTie1 or pFLAG-eTie2 protein. The gels were washed with binding buffer (50 mM Tris, 150 mM NaCl, 1 mM CaCl₂, pH 7.4), and then the culture supernatant containing recombinant Ang1, ARP1, or ARP2 was added to this gel. The gels were washed with clearance buffer (50 mM Tris, 150 mM NaCl, 1 mM CaCl₂, pH 7.4, 0.5% Triton X-100). The gels were boiled with sample buffer, and the samples were separated by 7.5% or 10% SDS-PAGE. Anti-FLAG-M2 antibody was used to detect the binding of the pFLAG-eTie1 and pFLAG-eTie2 proteins. Anti-Myc antibody was used to detect the binding of Ang1, ARP1, or ARP2.

**RESULTS**

**Cloning and Analysis of Human and Mouse ARP2—**We used degenerate PCR of a human heart cDNA to obtain a product with a novel sequence related to the angiopoietins. We used rapid amplification of cDNA ends-PCR to clone the remaining coding region and designated the gene ARP2. The human ARP2 cDNA encodes a 493-amino acid polypeptide. Mouse ARP2 was cloned by the same methods and shares 89% nucleotide identity with the human gene. The 493-amino acid mouse protein shares 95% amino acid identity with the human ARP2 product. ARP2 contains conserved fibrinogen-like domains in the COOH-terminal portion. According to the results from BlastP (NCBI) and ClustalW programs, the COOH-terminal portion of ARP2 is shared by a variety of other proteins, including the following: human fibrinogen-related protein-1 (11), fibrinogen A (12), CDT6 (angiopoietin-like factor from human cornea) (13), ficolin B (14), Ang2 (2), Hakata antigen (a member of the ficolin/opsonin p35 lectin family) (15), Ang1 (1), Ang4 (5), p35 (serum lectin) (16), fibrin B (17), and tenasin-R (GenBank access no. X98085). According to the ProteinPrediction program (EMBL), the NH2-terminal portion of ARP2 has coiled-coil domains, as do Ang1 and Ang2. The alignment of the deduced full amino acid sequence of ARP2 with those of ARP1, Ang2, and Ang1 is shown (Fig. 1A). Five of the nine cysteines in Ang1 are conserved in ARP2 (1). When gaps in the alignments are ignored in the calculations, ARP2 is 34% identical to Ang1 or Ang2, 35% identical to Ang3 or Ang4, and 59% identical to ARP1. The probable evolutionary relationships of the angiopoietin-related proteins were found using the ClustalW program (Fig. 1B). ARP2 is closest to ARP1. ARP1 and ARP2 are closer to the angiopoietin family protein than to fibrinogen or the lectin family.

**Expression of ARP2 in Human and Mouse Tissues, Rat Embryo, and Cultured Cells—**Northern blotting of human adult tissues revealed a major transcript of 4.0 kilobases, and a less-abundant transcript of 2.6 kilobases (Fig. 2A). Both ARP2 mRNAs were abundant in heart, small intestine, spleen, and stomach; they were both expressed less abundantly in colon, ovary, adrenal gland, skeletal muscle, and prostate. Among mouse tissues, the largest main transcript and the less abundant smaller transcript were present in most of the mouse adult tissues where ARP2 mRNA was detected (Fig. 2B). Although both ARP2 transcripts were abundant in heart, tongue, lung, and skeletal muscle, they were less abundant in the kidney, epididymus, and testis. Both sizes of ARP2 mRNA were abundantly in HUVECs and less abundantly in HUVSMCs, whereas they were not observed in HeLa and HepG2 cells (Fig. 3C). The result seen by *in situ* hybridization in rat embryo (E18) was consistent with that of the Northern blot analysis (Fig. 3). The ARP2 mRNA transcripts were abundant in the walls of the aortic trunk, pulmonary trunk, and descending aorta and were also present in the tongue, abdominal muscles, and back muscles (Fig. 3).
**Fig. 1.** Amino acids sequences and evolutionary relationships between ARP2 and its relatives. A, alignment of the deduced amino acid sequences of human ARP2 with human ARP1, human Ang2, and human Ang1. Residues that match the sequence of human ARP2 are shadowed. The filled circles above the human ARP2 sequence denote five cysteine residues that are conserved in Ang1 and Ang2. The line below the human ARP2 sequence marks the putative secretory signal sequence (13). Arrows limit the coiled-coil (large single arrows for ARP2 and ARP1; double arrows for Ang1) and fibrinogen like (small single arrows) domains. B, the evolutionary relationships (built by the ClustalW average distance tree using PID) of 13 proteins in the fibrinogen superfamily. The length of each horizontal line is proportional to the degree of amino acid sequence divergence.

**DISCUSSION**

Angiopoietin family proteins have characteristic protein structures that contain a coiled-coil domain in the NH$_2$-termi-

**ARP2 Is a Secreted Glycoprotein**—The amino acid sequence of human ARP2 has a highly hydrophobic region at the NH$_2$-terminus (~21 amino acids) that is typical of a signal sequence for protein secretion (Fig. 1A). The signal sequence cleavage site was predicted to lie between amino acid 21 (Ala) and 22 (Gly) using the method of von Heijne (19). To demonstrate that ARP2 is a secreted protein, COS-7 cells were transfected with CMV promoter-driven mammalian cell expression vector containing human ARP2 cDNA with a 3’-terminal extension encoding a c-Myc tag and a His$_6$ tag. As a positive control, human Ang1 cDNA was expressed in COS-7 cells using the same methods. To detect the ARP2 or Ang1 protein, both the culture medium and cell lysates were examined by Western blot analysis with an anti-Myc antibody (Fig. 4A). A major ARP2 band of ~64 kDa was detected in the culture medium but was barely present in the cell lysate (Fig. 4A). Similarly, a major Ang1 band of ~75 kDa was detected in the culture supernatant but was not present in the cell lysate. The observed molecular mass of the major ARP2 band was larger than its calculated molecular mass. The amino acid sequence of ARP2 contained two potential glycosylation sites, suggesting that ARP2, like Ang1, may be a glycoprotein. Therefore, the recombinant ARP2 and Ang1 were treated with PNGase-F (Fig. 4B). Deglycosylation reduced the apparent molecular mass of recombinant ARP2 from ~64 kDa to the predicted 57 kDa, whereas it reduced the apparent molecular mass of recombinant Ang1 from ~75 to 55 kDa (Fig. 4B). These results indicate that our recombinant ARP2 from transfected COS-7 cells is an efficiently secreted glycoprotein.

**ARP2 Induces Sprouting but Is Not Mitogenic in Endothelial Cells**—The inability of Ang1 and ARP1 to stimulate endothelial cell proliferation has been demonstrated (1, 4, 8). We examined the ability of ARP2 to stimulate endothelial cell proliferation by analyzing the incorporation of $[^3]$H/thymidine into HUVECs. Two hundred ng/ml of Ang1, ARP1, or ARP2 did not alter the incorporation of $[^3]$H/thymidine incorporation into DNA of HUVECs, whereas VEGF$_{165}$ (5 ng/ml) increased $[^3]$H/thymidine incorporation into DNA approximately 2.8-fold (Fig. 5A). These results demonstrate that ARP2, like Ang1, is not an endothelial cell growth factor in vitro. Ang1 is an effective inducer of sprouting in endothelial cells in vitro (9, 10). Therefore, we examined the sprouting activity of ARP1 and ARP2 in PPAEs, using VEGF$_{165}$ and Ang1 as positive controls (Fig. 5B). Although the control buffer produced a basal sprouting activity (approximate 3 sprouts per 50 MC beads), VEGF$_{165}$ (10 ng/ml) produced a 14.8 (~4.9)-fold increased sprouting activity compared with control (mean ± S.E.); Fig. 5C). Although Ang1 (200 ng/ml) produced an 8.4 (~2.6)-fold increase, ARP1 (200 ng/ml) and ARP2 (200 ng/ml) produced 3.4 (~0.8)- and 6.5 (~1.6)-fold increases, respectively.

**ARP1 and ARP2 Do Not Bind to Tie1 or Tie2**—Ang1 and Ang2 are secreted glycoproteins with considerable sequence homology (1, 2). Both bind to the Tie2 receptor with similar affinity, but neither binds to the closely related receptor Tie1 (1, 2). We assayed the binding activity of ARP1 or ARP2 with Tie1 and Tie2 by in vitro protein-protein interaction. The selected extracellular domain of Tie1 or Tie2, pFLAG-eTie1 (~96 kDa) or pFLAG-eTie2 (~105 kDa), respectively, was bound to an anti-FLAG affinity gel (Fig. 6A). As shown in Fig. 6B, recombinant Ang1 bound to pFLAG-eTie2, but not to pFLAG-eTie1, consistent with previous results (1, 2). However, neither ARP1 nor ARP2 bound to pFLAG-eTie2 or pFLAG-eTie1 (Fig. 6, C and D).
nal portion and a fibrinogen-like domain in the COOH-terminal portion (1, 2, 5). We have isolated a cDNA encoding a novel angiopoietin-related protein from human and mouse adult heart cDNAs and have designated it ARP2. The deduced amino acid sequences of human and mouse ARP2 reveal that the NH2-terminal and COOH-terminal portions of the protein also contain a characteristic coiled-coil domain and a fibrinogen-like domain, respectively. However, ARP2 is not an angiopoietin family protein because ARP2 has a low homology with Ang1 and Ang2 in the NH2-terminal portion, and ARP2 does not bind to either Tie1 or Tie2. According to analyses with the BlastP (NCBI), ClustalW, and ProteinPrediction (EMBL) programs, ARP2 is a member of the fibrinogen superfamily, which includes fibrinogens, angiopoietins, and lectins. We designated this protein as an angiopoietin-related protein for the following reasons: 1) the NH2-terminal portion of ARP2 has coiled-coil domains, like Ang1 and Ang2; 2) the evolutionary analysis indicates that this protein is closer to the angiopoietin family than to the fibrinogen or lectin families; and 3) this protein, like Ang1, induces sprouting in endothelial cells.

Like the angiopoietins and ARP1, ARP2 has a highly hydrophobic region at the NH2 terminus (–21 amino acids) that is...
typical of a signal sequence for protein secretion. The analysis and comparison of hydrophobicity profiles among Ang1, Ang2, ARP1, and ARP2 were performed using the Kyte and Doolittle algorithm (20). These results indicated that ARP2, like Ang1, Ang2, and ARP1, mainly consists of hydrophilic amino acids (data not shown) and is likely to be a secreted protein. Indeed, COS-7 cells transfected with CMV promoter-driven mamalian cell expression vector containing human ARP2 cDNA produced a major ARP2 protein band of ~64 kDa in the culture supernatant, but not in the cell lysate. Deglycosylation reduced the apparent molecular mass of the secreted recombinant ARP2 from ~64 kDa to the 57 kDa predicted by the sequence. In fact, the amino acid sequence of ARP2 contains two potential glycosylation sites. These results indicate that ARP2 is likely to be an efficiently secreted glycoprotein.

The expression patterns of the angiopoietin and ARP genes shed some light on their potential functions. ARP2 mRNA is widely expressed in adult tissues but is particularly abundant in adult muscle tissues, including heart, small intestine, and stomach. ARP1 mRNA is also widely expressed in the adult tissues, but it is expressed abundantly in highly vascularized glandular tissues, including the adrenal and thyroid glands (4). Ang1 mRNA is also widely expressed in adult tissues, although it is barely expressed in heart and liver (2). In contrast, Ang2 mRNA is expressed only in the ovary, uterus, and placenta, which are the three predominant sites of vascular remodeling in the normal adult (2). These findings suggest that different angiopoietin-related proteins may be required for maintenance.

Figure 5. Assays of recombinant protein activity in endothelial cells. A, the effect of recombinant proteins in [3H]thymidine incorporation into endothelial cells. HUVECs were incubated with control buffer (Cont), VEGF165 (5 ng/ml), Ang1 (200 ng/ml), ARP1 (200 ng/ml), or ARP2 (200 ng/ml), and incorporation of [3H]thymidine was measured. Columns represent the mean ± S.E. of the fold induction of [3H]thymidine incorporation compared with control. Results shown are the average of five independent experiments. Statistical analysis between the control and experiment was performed using one-way analysis of variance followed by the Student-Newman-Keuls test. Significant difference (p < 0.05) is marked with an asterisk. B, representative phase-contrast photographs of sprouting activity of PPAECs. Cells grown on MC beads were placed in fibrin gels containing control buffer, recombinant VEGF165 (10 ng/ml), Ang1 (200 ng/ml), ARP1 (200 ng/ml), or ARP2 (200 ng/ml) protein and were incubated in M-199 with daily supplementation with same amount of recombinant protein. After 3 days, the extent of sprouting was determined using a phase-contrast inverted microscope. Magnifications are × 200. C, quantification of the sprouting activities. The number of endothelial sprouts with length exceeding the diameter (175 μm) of the MC bead was determined for every 50 MC beads counted as described (9). Columns represent the mean ± S.E. from five independent experiments. Statistical analysis was as noted above.

Figure 6. In vitro protein-protein interaction between ARP1, ARP2, or Ang1 and pFLAG-eTie1 or pFLAG-eTie2. A, control experiment shows that the pFLAG proteins bind to the anti-FLAG affinity gel. After binding of pFLAG-eTie1 or pFLAG-eTie2, the gels were boiled with sample buffer, separated by SDS-PAGE, and probed with anti-FLAG antibody. Note that an equal amount of pFLAG-eTie1 or pFLAG-eTie2 protein is present in the gel. B–D, after binding of Ang1, ARP1, or ARP2 to pFLAG-eTie1- or pFLAG-eTie2-coupled gels, the gels were boiled with sample buffer, separated by SDS-PAGE, and probed with anti-Myc antibody. Note that Ang1 binds to pFLAG-eTie2, but not to pFLAG-eTie1, whereas ARP1 and ARP2 do not bind to either protein. Results were similar in three independent experiments.
of the differentiated state of endothelial cells or for vascular remodeling in adult tissues (1, 2, 4). Angiogenesis and vasculogenesis are active during development. In mid-gestational mouse embryos, Ang1 mRNA transcripts are abundant in the heart and in the mesenchymal and smooth muscle cells surrounding most blood vessels, including the dorsal aorta and vessels of neural tissues, the somites, and lung (2). In contrast, Ang2 mRNA transcripts are not readily detected in the developing heart but are abundant in the dorsal aorta and major aortic branches, specifically in the smooth muscle layer beneath the vessel endothelium (2). In situ hybridization analysis demonstrated that ARP2 mRNA is abundant in embryonic muscle tissues, including those of the aortic trunk, pulmonary trunk, descending aorta, tongue, abdomen, and back. The distinct but overlapping expression patterns of Ang1, Ang2, and ARP2 are consistent with the possibility that these proteins may regulate vascular development at particular sites and stages with different unique functions.

The Tie family of receptors consists of two members, Tie1 and Tie2, or TEK (21). Tie1 and Tie2 are single transmembrane receptor tyrosine kinases. Tie1 and Tie2 are expressed predominantly in vascular endothelial cells and some hematopoietic cells (22, 23). Mice with a targeted disruption of the Tie1 gene die between E14.5 and birth. Death is associated with edema and hemorrhage, suggesting that the Tie1 signals control fluid exchange across capillaries and hemodynamic resistance (24, 25). Mice with targeted disruption of the Tie2 gene die between E9.5 and E10.5. In these mice, the normal number of endothelial cells are present, but the blood vessels are immature, lacking branching networks and proper organization into large and small vessels (18, 24). Thus, Tie2 may control the ability of endothelial cells to recruit stromal cells to encase the endothelial tubes to stabilize the structure and modulate the function of blood vessels. Ang1 and Ang2 are ligands for Tie2 and bind to it with similar affinity (1, 2). In vivo analysis by targeted gene inactivation reveals that Ang2 is a naturally occurring antagonist of Ang1. Ang2 competes for Ang1 binding to Tie2 and blocks Ang1-induced Tie2 autophosphorylation (2). Both Ang3 and Ang4 bind to Tie2 but not to Tie1 (5). Moreover, Ang4, like Ang1, is an agonist for the Tie2 receptor, whereas Ang3, like Ang2, is an antagonist for Tie2 (5). However, ligands for Tie1 have not been identified. Given the similarity between the angiopoietins and the ARPs, we speculated that ARP1 or ARP2 is a glycosylated secretory protein that induces sprouting in endothelial cells through an autocrine or paracrine action.

In summary, we have isolated a novel angiopoietin-related protein and have designated it ARP2. ARP2 contains the characteristic coiled-coil domains and fibrinogen-like domain found in angiopoietins but has only modest overall homology to them. ARP2 mRNA is most abundant in adult muscle tissues, including heart, small intestine, tongue, and stomach. During development, the expression patterns of ARP2 are overlapping, but distinct from, those of Ang1 and Ang2. ARP2 is a glycosylated secretory protein that induces sprouting in endothelial cells through an autocrine or paracrine action.

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