Developmental Biology 448 (2019) 309–319

Contents lists available at ScienceDirect

Developmental Biology

journal homepage: www.elsevier.com/locate/developmentalbiology

Review article

The biology of the extracorporeal vasculature of *Botryllus schlosseri*

Delany Rodriguez*, Shane Nourizadeh, Anthony W. De Tomaso

Department of Molecular, Cellular, and Developmental Biology, University of California Santa Barbara, Santa Barbara, CA 93106, USA

ARTICLE INFO

Keywords:
Invertebrate-vasculature
Extracorporeal-vasculature
Vascular regeneration
Vascular regression
Angiogenesis
Parabiosis

ABSTRACT

The extracorporeal vasculature of the colonial ascidian *Botryllus schlosseri* plays a key role in several biological processes: transporting blood, angiogenesis, regeneration, self-nonself recognition, and parabiosis. The vasculature also interconnects all individuals in a colony and is composed of a single layer of ectodermally-derived cells. These cells form a tube with the basal lamina facing the lumen, and the apical side facing an extracellular matrix that consists of cellulose and other proteins, known as the tunic. Vascular tissue is transparent and can cover several square centimeters, which is much larger than any single individual within the colony. It forms a network that ramifies and expands to the perimeter of each colony and terminates into oval-shaped protrusions known as ampullae. *Botryllus* individuals replace themselves through a weekly budding cycle, and vasculature is added to ensure the interconnection of each new individual, thus there is continuous angiogenesis occurring naturally. The vascular tissue itself is highly regenerative; surgical removal of the ampullae and peripheral vasculature triggers regrowth within 24–48 h, which includes forming new ampullae. When two individuals, whether in the wild or in the lab, come into close contact and their ampullae touch, they can either undergo parabiosis through anastomosing vessels, or reject vascular fusion. The vasculature is easily manipulated by direct means such as microinjections, microsurgeries, and pharmacological reagents. Its transparent nature allows for in vivo analysis by bright field and fluorescence microscopy. Here we review the techniques and approaches developed to study the different biological processes that involve the extracorporeal vasculature.

1. Introduction

Tunicates are marine invertebrate chordates considered the closest living non-vertebrate to vertebrates (Delsuc et al., 2006; Kocot et al., 2018). *Botryllus schlosseri* belong to the polyphyletic sub-phylum Tunicata (tunicates), class Ascidiacea (ascidians), and family Styelidae; a family where both solitary and colonial organisms have been identified (Alie et al., 2018; Brown and Swalla, 2012; Delsuc et al., 2018; Gasparini et al., 2015; Holland, 2016). Ascidians can reproduce both sexually and asexually (Fig. 1B) (Brown and Swalla, 2012; Gasparini et al., 2015; Rodriguez et al., 2017b, 2014). *B. schlosseri* embryonic development results in a tadpole larva with typical chordate characteristics, but then undergoes metamorphosis to give rise to a sessile invertebrate known as an oozooid. This initial oozooid then expands the colony through a lifelong asexual budding process termed blastogenesis, in which genetically identical zooids are added (Fig. 1A). Each adult individual has a fully functional heart, gastrointestinal tract, nervous system, and germline.

The *B. schlosseri* vascular system is a unique combination of two disparate structures. First, within each zooid exists an open circulatory system comprised of: a tubular heart, lacunae, and tissues bathed in blood (Burighel and Brunetti, 1971; Gasparini et al., 2008). Second, a large ramified transparent extracorporeal vasculature forms a net of monolayered vessels that extend out to the periphery of the colony and terminate in specialized structures known as ampullae (Fig. 1C and D). Ampullae are protrusions that adhere to a substrate and are also involved in other biological processes as discussed later (Fig. 1E and F). They are not all elongated and positioned at the periphery of the colony, but are also found in other regions of the extracorporeal vasculature where they are spherical. The intricate network of anastomized blood vessels, including ampullae and zooids, are fully embedded in an extracellular matrix (ECM) known as the tunic. This tunic has a composition that includes four main macromolecules: collagens, proteoglycans, glycoproteins, and cellulose (Gasparini et al., 2015; Matthysse et al., 2004; Patricolo and Ferrarella, 1973; Wei et al., 2017). Many cells are also found within the tunic; some that are directly in contact with the blood vessels and others that are highly mobile (Fig. 1D) (Ballarin et al., 2008). In *B. schlosseri*, the tunic is thin and transparent, which allows for easy observation and manipulation of the vasculature (Gasparini et al., 2015; Zaniolo, 1981, 1987).

* Corresponding author.
E-mail address: drodriguez@lifesci.ucsb.edu (D. Rodriguez).

https://doi.org/10.1016/j.ydbio.2018.10.013
Received 28 March 2018; Received in revised form 29 September 2018; Accepted 17 October 2018
Available online 25 October 2018
0012-1606/

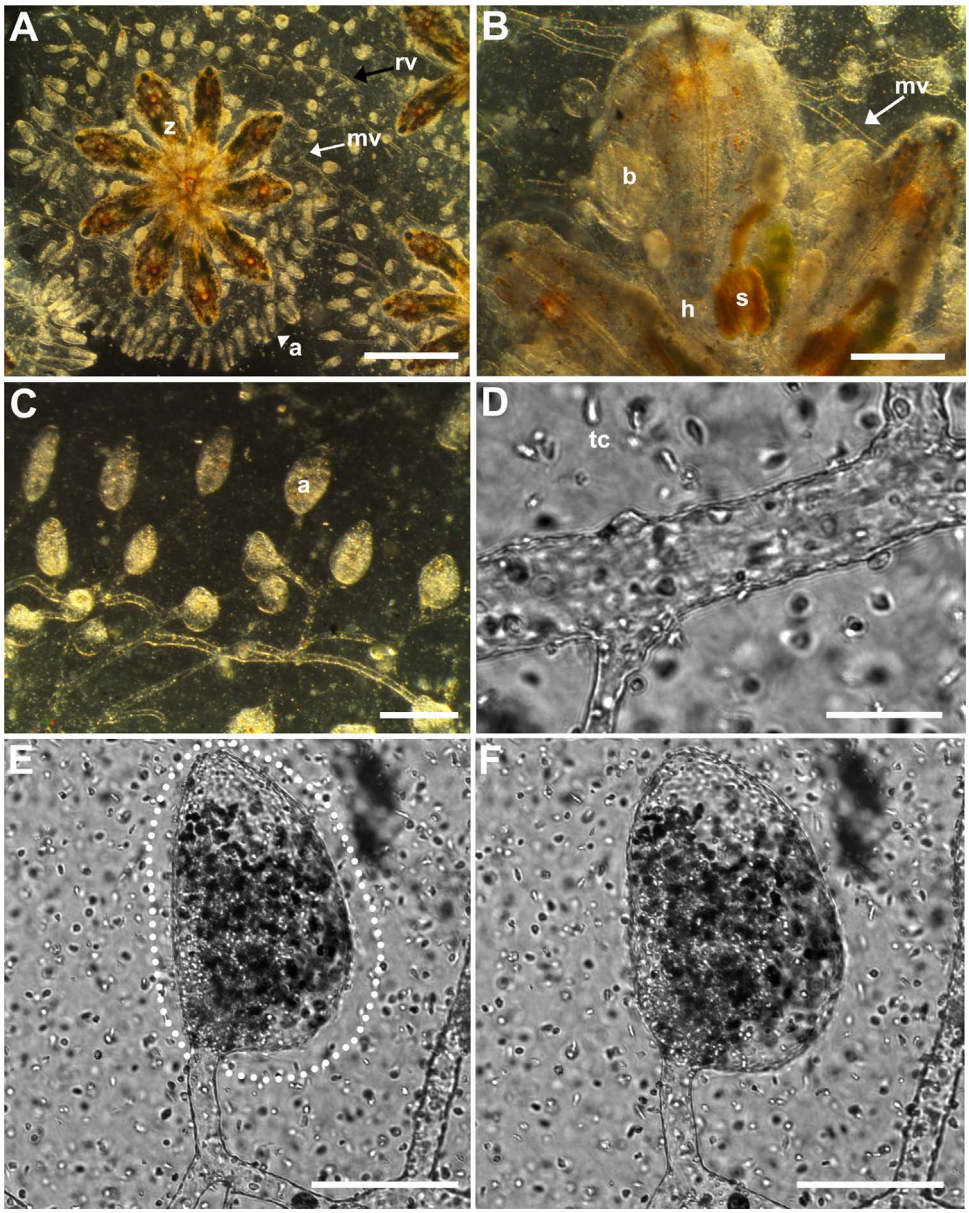

Fig. 1. The extracorporeal vasculature of *Botryllus schlosseri*. A. Light micrograph of a *Botryllus schlosseri* colony; the zooids (z) arrange themselves into star-shaped structures called systems. A colony can consist of multiple systems, all interconnected by a large common extracorporeal vasculature. One main blood vessel (mv) directly connects all individuals. From the mv the vasculature extends out (radial vessel (rv)) to the periphery of the colony and terminates into pouch-like structures called ampullae (a). B. Close up light micrograph of a zooid. Although the lacunae and sinuses in the body are not easily visualized, organs such as the heart (h) and stomach (s) are easy to identify as well as the asexually reproducing bud (b). C. A close up view of the periphery of the colony showing ampullae. D. Zoom-in of a blood vessel embedded in the tunic and showing tunic cells (tc). E and F. Zoom-in of a contracting and relaxing ampullae, respectively (dotted line on E provides comparison). Scale Bars: A) 2 mm, B) 1 mm C) 500 μm D) 100 μm E and F) 50 μm.

The extracorporeal vasculature, also described as the colonial circulatory system (Gasparini et al., 2007), can be divided into four components: 1) marginal/main vessel, 2) radial/peripheral vessels, 3) ampullae, and 4) an intricate network of anastomosed blood vessels that spread around the vascular bed and connect components 1–3. The main vessel is a continuation of the zooid's open circulatory system, and each zooid has at least two epidermal connections to the extracorporeal vasculature: one directly to the main vessel, and a second one to the peduncular vessel (Brunetti, 1969). The radial vessel connects to each zooid, including primary buds but excluding secondary buds, to the surrounding vasculature. It is formed by the growth and fusion of two converging close-ended extrusions, one from the zooid epidermis and the other from a nearby vessel (Fig. 1A). Peripheral ampullae are sac-like structures delimited by a monolayer epithelium that is columnar, but only at the tip of the leading edge ampullae. Ampullae are involved in adhesion and are contractile enough to collectively provide continuous blood flow (Fig. 1E and F; discussed below). All of the components of the extracorporeal vasculature are highly regenerative and characterized by their ability to expand with angiogenic-like properties (Braden et al., 2014; Gasparini et al., 2008, 2014, 2007; Tiozzo et al., 2008).

2. Similarities and difference with vertebrate vasculature

2.1. Vertebrate vasculature

Blood vessels are tubular structures that transport blood throughout the body, and are formed by two mechanisms: vasculogenesis and angiogenesis (Flamme et al., 1997; Risau and Flamme, 1995; Risau et al., 1988). During vasculogenesis, precursor cells are differentiated into endothelial cells that give rise *de novo* to a primitive vascular network formation (Risau and Flamme, 1995). In contrast, angiogenesis is defined

as the formation of new blood vessels from preexisting ones (Vailhe et al., 2001). Vertebrate tubule formation initiates from a simple epithelial pocket that sprouts branches to extend into a web-like network of interconnected tubes (Davies, 2002, 2005; Lubarsky and Krasnow, 2003). Defects in tube formation often lead to major disorders, one example being ductal plate malformations (Strazzabosco and Fabris, 2012). Tubular structures are diverse in size, shape, and origin, yet they have a common characteristic; they are a wrapped epithelium with the cells apical surface lining the lumen. Vertebrate blood vessels are lined on the inside with mesodermally derived endothelial cells (Onat et al., 2011; Shoda et al., 2016; Zecchin et al., 2017). They are externally layered by a basement membrane (BM) that is further surrounded by smooth muscle. The vessel morphology is characterized by having the apical side of the cell surfaces lining the lumen, and BM and smooth muscle cells located abluminal (Lubarsky and Krasnow, 2003). Endothelial cells are essential for vertebrate angiogenesis because they express vascular endothelial growth factor (VEGF) and angiopoietin receptors that control this process. Angiogenesis first takes place during embryogenesis, and continues to occur throughout life to provide regeneration (e.g. injury), renewal conditions (e.g. menstruation), and also unwanted tumor vascularization (Breier et al., 1997; Flamme et al., 1997; Hickey and Fraser, 2003; Treps and Gavard, 2015; van der Bilt and Borel Rinkes, 2004; Xu et al., 2007). Vertebrate angiogenesis first requires the degradation of BM followed by: extension of exploring filopodia, proliferation, endothelial cell migration towards angiogenic stimuli, secretion of new BM, recruitment of perivascular cells, and finally lumen formation. All of these biological processes are regulated by VEGF (Chavez et al., 2016; Craig and Sumanas, 2016; Schittny, 2017). VEGF stimulus causes a local degradation of the BM, is followed by cell migration, and leads to the formation of blind tubes (Kalluri, 2003; Risau, 1997). The most common mechanism of vertebrate angiogenesis is sprouting, which usually occurs in early processes such as retinal vasculature formation during eye development (Fruttiger, 2002; Gariano, 2003; Gerhardt and Betsholtz, 2005; Weinstein, 2005; Zacchigna et al., 2008). The other type of angiogenesis is known as intussusceptive, or splitting angiogenesis, and is poorly understood. In general, it refers to the formation of new blood vessels by splitting existing ones into two (De Spiegelaere et al., 2012; Makanya et al., 2009).

2.2. *Botryllus* vasculature

It has been hypothesized that endothelial cells lining the blood vessels of vertebrates evolved from invertebrate hemocytes sporadically binding to the BM of invertebrate blood vessels (Strilic et al., 2010). In *B. schlosseri,* the blood vessels are a simple squamous epithelium whose apical side faces the tunic, and basal side along with BM faces the lumen (Fig. 2). These blood vessel cells are also myoepithelial and can contract. The tissue architecture with the BM directly facing the lumen and blood, allows circulating cells to directly attach to the BM. Evolutionarily it can be postulated that amoebocytes, or a similar cell type, are the cells that endothelial cells derived from.

Botryllus vascular cells are characterized by having apicolateral tight junctions (Gasparini et al., 2007), and utilizing angiogenic growth factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), epidermal growth factor (EGF) and receptors (VEGFR-1, VEGFR-2, and EGFR). Gasparini et al. (2007) showed that antibodies against vertebrate angiogenic growth factors and receptors can cross-react and detect *Botryllus* protein homologs. These antibodies showed positive signal in regenerating regions of the vessel epithelium and were also detected at the active apex of peripheral ampullae. Interestingly, the transcript for VEGFR was detected, using fluorescent in situ hybridization (FISH), in blood vessels and ampullae, but not in the epithelium lining the lacunae and sinuses of the zooids (Fig. 3) (Braden et al., 2014; Tiozzo et al., 2008). In contrast to the antibody staining, FISH revealed a positive signal for all vascular cells including ampullae and potentially some tunic cells. The abundance discrepancy between mRNA transcripts and proteins has been observed before and it is understood that these two do not always correlate or have the same spatial localization (Wang, 2008). *Botryllus* vascular cells, however, do seem to have high levels of BsVEGFR mRNA, perhaps indicating that these cells have the ability to rapidly respond to VEGF stimulus for tissue repair and remodeling.

Botryllus vascular cells express vascular progenitor cells markers such as CD133 and VEGFR-2, and a marker for differentiated vasculature, VE-Cadherin (Braden et al., 2014). To study the role of BsVEGFR during homeostasis, siRNA was administered for three weeks. During the first two weeks, the budding and physiology of the colony appeared normal. At the beginning of the third week the typical star-shaped zooid organization became disorganized, and the extracorporeal vasculature became leaky. Blood cells started to spill into the tunic while the ampullae became deflated, and the overall tunic appearance changed from transparent to opaque (Tiozzo et al., 2008). In conclusion, BsVEGFR plays a key role regulating homeostasis in the extracorporeal vasculature.

It has been shown that some types of vertebrate tumors have blood spaces that are not lined by an epithelium. These tumors can trigger *de novo* vessel formation through a poorly understood mechanism known as vasculogenic mimicry (Folberg and Maniotis, 2004; Kucera and Lammert, 2009). These vessels are capable of distributing plasma and blood, thereby mimicking the function of normal vessels (Folberg and Maniotis, 2004). Tumors displaying vasculogenic mimicry have been shown to upregulate genes involved in angiogenesis and vasculogenesis (Paulis et al., 2010). It has been suggested that novel and potentially useful anticancer therapies can be found by studying the molecular mechanisms of invertebrate vascular tube formation given its similarities between vasculogenic mimicry in tumor growth (Gasparini et al., 2014; Kucera and Lammert, 2009).

The two major differences between invertebrate and vertebrate blood vessels are the presence of the vertebrate endothelial cell layer, and that *Botryllus* extracorporeal vasculature growth does not requires degradation of a BM. The similarities between angiogenesis, however, are found in the signaling pathways controlled by VEGF and VEGFR. Because of these differences and similarities it has been suggested to use the terms “endothelial angiogenesis” when referring to vertebrates and “myoepithelial angiogenesis” when referring to invertebrates (Munoz-Chapuli, 2011; Munoz-Chapuli et al., 2005).

3. Angiogenesis (natural expansion of the colony)

Angiogenesis has been defined in vertebrates as the process where new blood vessels are formed from preexisting ones. This includes the process of remodeling and expansion of blood vessels in both physiological and pathological conditions such as recovering from trauma/surgery, and the vascularization of tumors, respectively (DiPietro, 2016; Rocha et al., 2018). Angiogenesis starts *in utero* and continues through the adult life of an individual (Walls et al., 2008). For example, vessel regression is normally observed during luteolysis, and the involution of the mammary gland (Guzman et al., 2015; Modlich et al., 1996; O'Brien et al., 2010; Zarzynska and Motyl, 2008). Defects in angiogenesis can lead to diseases such as age-related wet macular degeneration, diabetic retinopathy, and cancer (Watson et al., 2017).

In *Botryllus*, the extracorporeal vasculature propagates by extending the vessel network through the tunic in a mechanism similar to vertebrate angiogenic sprouting (Brunetti, 1969; Burighel and Brunetti, 1971; Gasparini et al., 2008, 2014, 2007; Tiozzo et al., 2008). As new individuals are added, the extracorporeal vasculature adapts and grows to meet the demands of a larger colony. The natural expansion of the extracorporeal vasculature starts with a thickening of the vessel wall which will either form a blind tube to fuse with other vessels, or differentiate into an ampullae (Braden et al., 2014; Gasparini et al., 2008, 2014, 2007). During the expansion process, blood vessels are formed to connect newly developing buds (Gasparini et al., 2007). Ampullae development starts behind the outer-ring of

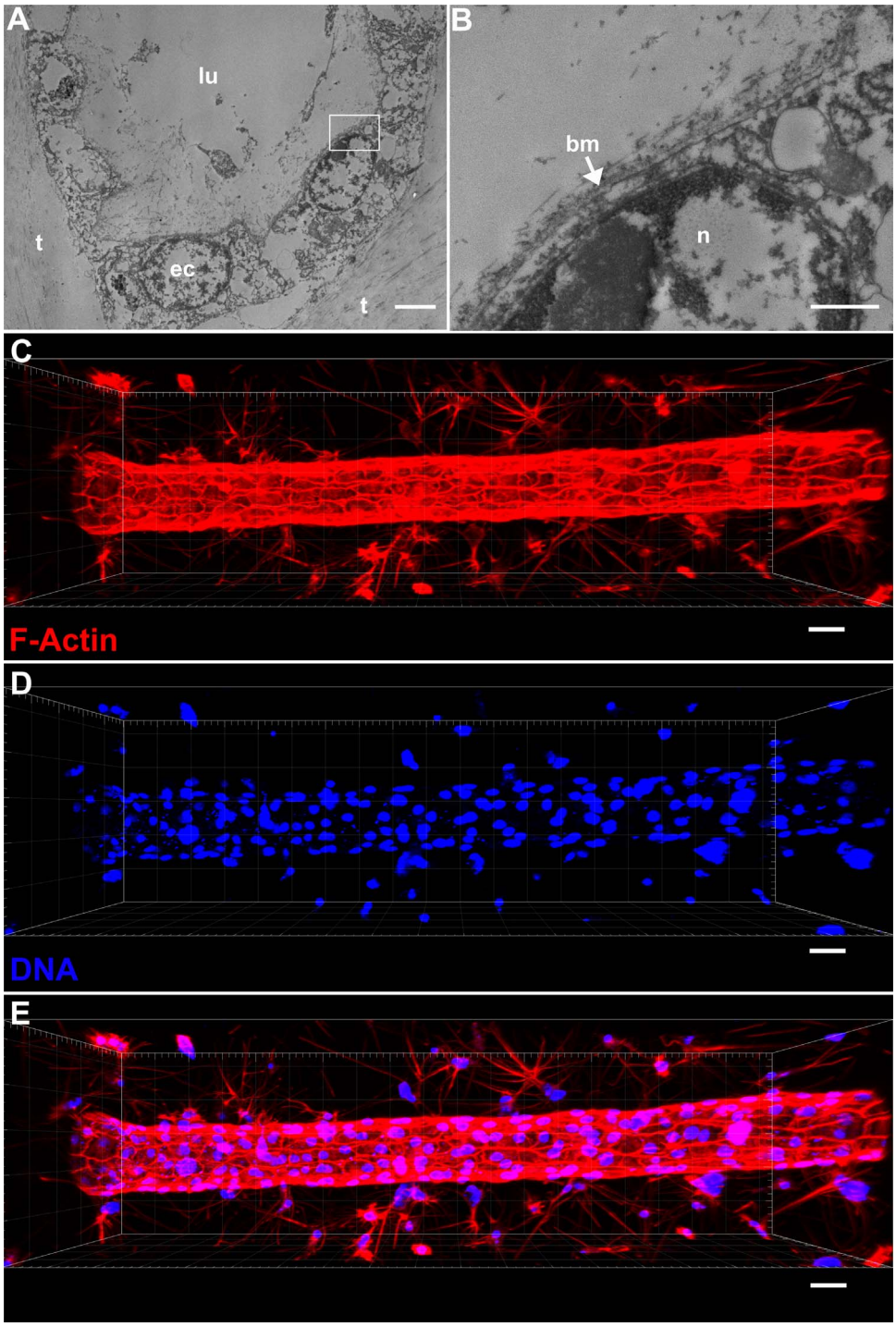

Fig. 2. Architecture of a *Botryllus* blood vessel. A. Transmitted Electron Micrograph of frontal plane of a blood vessel with the lumen (lu) in the center surrounded by a monolayer of endothelial cells (ec), and the tunic (t) directly outside. B. A zoom-in from A (white frame) depicting an endothelial cell with the basement membrane (bm) facing the lumen right across from the nucleus (n). C-E. Projection of a confocal z-stack of a blood vessel stained with Phalloidin (C) and DAPI (D) and the merged imaged of both (E). Scale bars A) 200 nm B) 500 nm C, D, E) 50 μm.

existing ampullae, and then elongates outward to the same extent. The apex of each ampullae use filopodia to extend into the tunic. Cells of the extending ampullae are characterized by their basal nucleus, and by having membrane bound secretory vesicles discharging their contents directly into the tunic. It has been observed by transmitted electron microscopy (TEM) that cells on sprouting apexes undergo epithelial-to-mesenchymal transition during ampullae formation, stop proliferation and migration, and retain their capability to produce tunic (Gasparini et al., 2007).

4. Vascular regression

Angiogenesis is often thought of as the growth of blood vessels; however, it includes and is balanced by vascular regression in order to prune excess vessels formed during growth (Korn and Augustin, 2015). It has been shown that milk-producing breast mammary glands undergo vascular regression after weaning (McNally and Stein, 2017; Silanikove, 2014), so to ensure blood supply for constantly changing tissues, a homeostatic combination of blood vessel growth and regres-

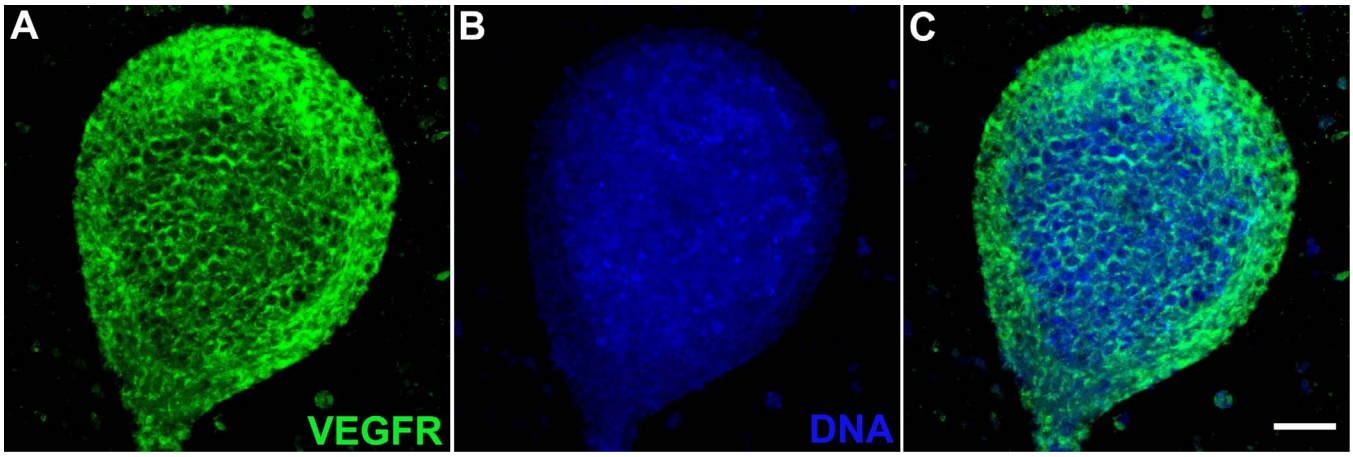

Fig. 3. Fluorescent in situ hybridization of BsVEGFR. A. Expression of *BsVEGFR* on ampullae, riboprobe shown in green. B. Same ampullae counter stained with DAPI shown in blue. C. Merge image from A and B. Scale bar 25 μm.

sion is necessary (Sweat et al., 2017). Induced vascular regression is a major target for new anti-angiogenic related drugs and therapies to treat vascularized tumors (Ribatti, 2009; Ricciuti et al., 2017a, 2017b).

At the end of each budding cycle in *Botryllus*, zooids undergo a massive apoptotic event and their corpses are engulfed by blood borne phagocytes (Chang and Lauzon, 1995; Cima et al., 2003; Franchi et al., 2016a, 2016b; Lauzon et al., 1993; Tiozzo et al., 2006; Voskoboynik et al., 2004). This event is crucial to allow the new zooids to occupy the vacant space of the previous generation (Ballarin et al., 2010; Campagna et al., 2016; Cima et al., 2010; Lauzon et al., 1992, 2007, 2002; Rinkevich et al., 1992). During this process, termed "take over", there is a massive rearrangement of the extracorporeal vasculature. It is characterized by regression of all blood vessels as the zooid corpses are pulled to the center of each system, followed by expansion to allow more space for new zooids and buds (Lauzon et al., 2002). This can be considered as a natural regression followed by a natural expansion of the extracorporeal vasculature.

Recently, our group has shown by FISH that lysyl oxidase 1 (LOX1) homolog in *Botryllus* is expressed by vascular cells (Rodriguez et al., 2017a). LOX1 is involved in the cross-linking of collagen and elastin, is secreted by blood vessels (Rodriguez et al., 2008), and is crucial for ECM stability and remodeling. Through pharmacological inhibition of LOX activity using BAPN, a specific small molecule inhibitor, we were able to manipulate the stiffness of the BM, which caused vascular regression of the entire extracorporeal vasculature within 16 h (Fig. 4A and B). This coordinated blood vessel regression maintaining blood flow without bleeding, and displayed a disrupted collagen arrangement. A 10-fold increase in apoptotic cells was observed in regressing vessels; however, only a subset of vascular cells become apoptotic rather than the entire vascular tissue. This type of apoptosis, termed anoikis, where programmed cell death is induced by an improper or lack of attachment to the ECM (Taddei et al., 2012). Apoptotic cells are extruded directly into the lumen while being ingested by circulating phagocytes; blood-borne phagocytes are key in removing cells that are extruded basolaterally across the BM during induced vascular regression. Further analysis of the integrin pathway by inhibition of Focal Adhesion Kinase (FAK) showed similar vascular regression patterns to LOX 1 inhibitor, and BAPN regressing colonies did show low levels of FAK phosphorylation when compared to controls. Our results suggest that disruption of collagen crosslinking induces cells to undergo anoikis due to their lack of integrin-mediated binding to the disrupted ECM.

5. Vascular regeneration upon injury

Botryllus extracorporeal vasculature can completely regenerate vessels that have been injured through damage or surgical removal (Fig. 4 C and D). To study vascular regeneration, the vasculature and peripheral ampullae are surgically removed to leave behind only the main vessel. This type of surgery is known as an ampullectomy (Braden et al., 2014; Gasparini et al., 2008; Tiozzo et al., 2008). It has been shown that *Botryllus* blood vessel angiogenesis can be induced by injecting human pro-angiogenic growth factors, VEGF and EGF, into colonies following ampullectomies (Gasparini et al., 2014). Upon surgical removal of ampullae and radial vessels, blood leakage occurs for five to ten seconds until blood cells clot at sites of injury to stop the bleeding (Gasparini et al., 2008, 2014; Tiozzo et al., 2008). Blood flow then returns to normal about one-hour post-surgery. In the case that the extracorporeal vasculature is partially removed (i.e. only half) it has been observed that the remaining ampullae reposition toward the site of surgery. The apexes of these ampullae become active and in some cases penetrate the regenerating area to aid in the regrowth process (Gasparini et al., 2008). Newly formed ampullae can be observed around 24 h after surgical ablation (Braden et al., 2014; Gasparini et al., 2008, 2014; Tiozzo et al., 2008). The post-surgery vessel stumps become actively involved in vessel regeneration, and new vessels will also form from branching points or start as sprouts that rapidly bifurcate from the main vessel.

Some cells detach from the active apex of sprouts and migrate into the tunic. Detaching cells change their tight junctions from apicolateral to basal position while their neighbors maintain apicolateral. The apical surface of a detaching cell extends out filopodia into the tunic, and as this cell protrudes, it detaches and leaves the lumen intact. As the detaching cells moves out, it maintains basal tight-junctions with neighbors, and neighbors eventually converge and restore apicolateral tight junctions providing continuous integrity to the BM (Gasparini et al., 2008).

The regeneration of the extracorporeal vasculature has been classified into five stages: 1) blood clotting followed by small bulb formation, 2) bulb formation growth and becoming round in shape, 3) the structure becomes oval, 4) the main vessels have completed regeneration and are actively extending newly formed ampullae, and 5) the ampullae gain back their average size.

In adult colonies it takes 3–7 days to fully regenerate the vessel network to the same extent as before surgery (Braden et al., 2014; Gasparini et al., 2008, 2007; Tiozzo et al., 2008). In contrast, an oozooid can regenerate all ampullae in about 24 h (Tiozzo et al., 2008); however, it is important to note that in the case of an oozooid, the ampullae are proximal to the zooid and do not have expanded vasculature. Therefore, young colonies containing 2–5 zooids with expanded extracorporeal vasculature may take longer approximately 3–7 days, to fully regenerate.

siRNA-mediated knockdown of VEGFR prevented regeneration and formation of new blood vessels and ampullae after ampullectomy

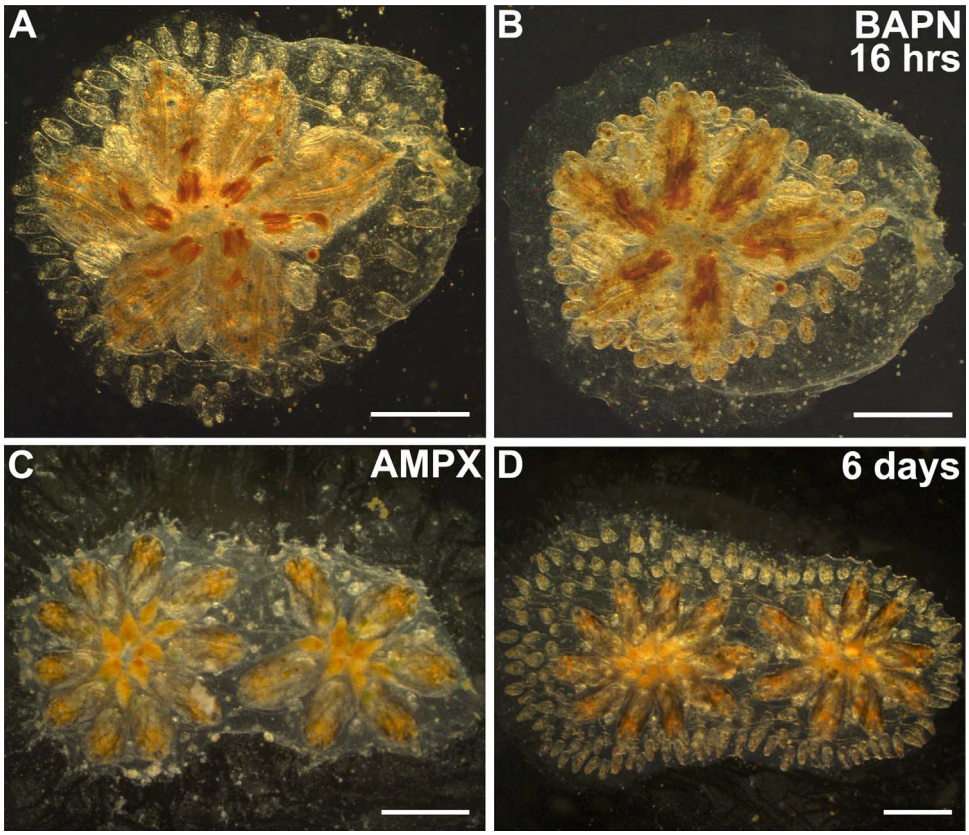

Fig. 4. Vascular regression and regeneration. A. Bright field image of a young colony before induced regression. B. Same colony as after induced vascular regression by incubation of BAPN for 16 h. C. Bright field image of a colony right after ampullectomy (all the extracorporeal vasculature and ampullae have been removed except for the vessels interconnecting zooids). D. Same colony as C 6 days after ampullectomy; all newly regenerated extracorporeal vasculature has extended out to the same area as before the surgery. Scale bars A-D 2 mm.

(Tiozzo et al., 2008). The zooids did not present abnormalities; they continued budding, and blood flow was present. In contrast, in the BsVEGFR knockdown without ampullectomy, the zooids lost their characteristic star-shaped association and presented a random position in the colony (Tiozzo et al., 2008). The VEGFR siRNA results were phenocopied using a pharmacological inhibitor of VEGF receptors (PTK787), which was able to inhibit both vessel and ampullae regeneration for 120 h post-ampullectomy. The authors concluded that the extracorporeal vasculature serves as an important cue to modulate the spatial organization and distribution of the zooids in a colony. Similarly, mouse pancreas organogenesis requires growth factors to dictate apicobasal polarization to maintain proper tissue organization and architecture (Lof-Ohlin et al., 2017; Shih et al., 2013). During pancreas organogenesis, individual cells acquire apicobasal polarity with the apical side facing the lumen, and the basal side attached to the ECM. These polarized cells form rosettes that fuse into a luminal plexus, that in-turn forms a tubular monolayer epithelium attached to a basement membrane (reviewed in Shih et al., 2013). It has been shown that EGFR is capable of modulating both morphogenesis and cell differentiation in a context specific manner, thereby modulating apical polarity through pancreatic organogenesis (Lof-Ohlin et al., 2017).

Braden et al. (2014) developed a novel vascular lineage tracing method that was also used as a marker for isolating vascular cells. Lineage tracing was obtained taking advantage of proteins, in this case Bovine Serum Albumin, bound to a pH stable Alexa Fluor that can maintain fluorescence inside the low-pH lysosomal environment (Fig. 5). Cells that engulf these pH stable protein-bound fluorophores undergo proliferation and equally partition the number of lysosomes between daughter cells, therefore maintaining the label. The molecular mechanism underlying specific uptake of this conjugate into *Botryllus* vascular cells is not well understood, yet it is clear that *Botryllus* vascular cells intake and retain it, which serves as a cell linage tracing tool. Using this label, it is possible to study the role of vascular epithelial cells in vascular regeneration, proliferation, mobility, and differentiation into ampullae. Braden et al. (2014) also showed that regenerative resident cells within the vascular tissue have the potential to form newly regenerated blood vessels and differentiated ampullae, and that *Botryllus* blood vessel regeneration occurs through a combination of proliferation and differentiation. Only a few studies have addressed the molecular mechanism underlying regeneration of blood vessels in *Botryllus*.

In mice, mutation of Vascular Endothelial (VE) Cadherin is lethal due to vascular defects during embryogenesis (Carmeliet et al., 1999; Gory-Faure et al., 1999). These studies concluded that VE-Cadherin plays a key role in the survival of endothelial cells involved in embryo vascularization. CD133, also known as Prominin-1, is a stem cell/progenitor marker expressed at the apical side of several tissues and may play a role in regeneration of tubular epithelial after injury (Corbeil et al., 2014; Jang et al., 2017; Karbanova et al., 2014; Kramann et al., 2015; Kusaba et al., 2014). It has been shown that injury to ampullae causes a local up-regulation of Bs-Cadherin in damaged tissue similar to how endothelial VE-Cadherin responds to injury of blood vessels (Rosner et al., 2007; Wallez et al., 2006). CD133 is expressed by regenerating ampullae, which is in accordance with its importance in regulating branching morphogenesis (Anderson et al., 2011; Braden et al., 2014). It has been proposed that CD133 may have a role in ampullae differentiation (Braden et al., 2014).

6. Self and non-self recognition

Several reviews have thoroughly covered this process (De Tomaso, 2006; McKitrick and De Tomaso, 2010; Rinkevich, 1996, 2002, 2004; Rosengarten and Nicotra, 2011; Taketa and De Tomaso, 2015). Ampullae are implicated in adherence of the colony to a substrate,

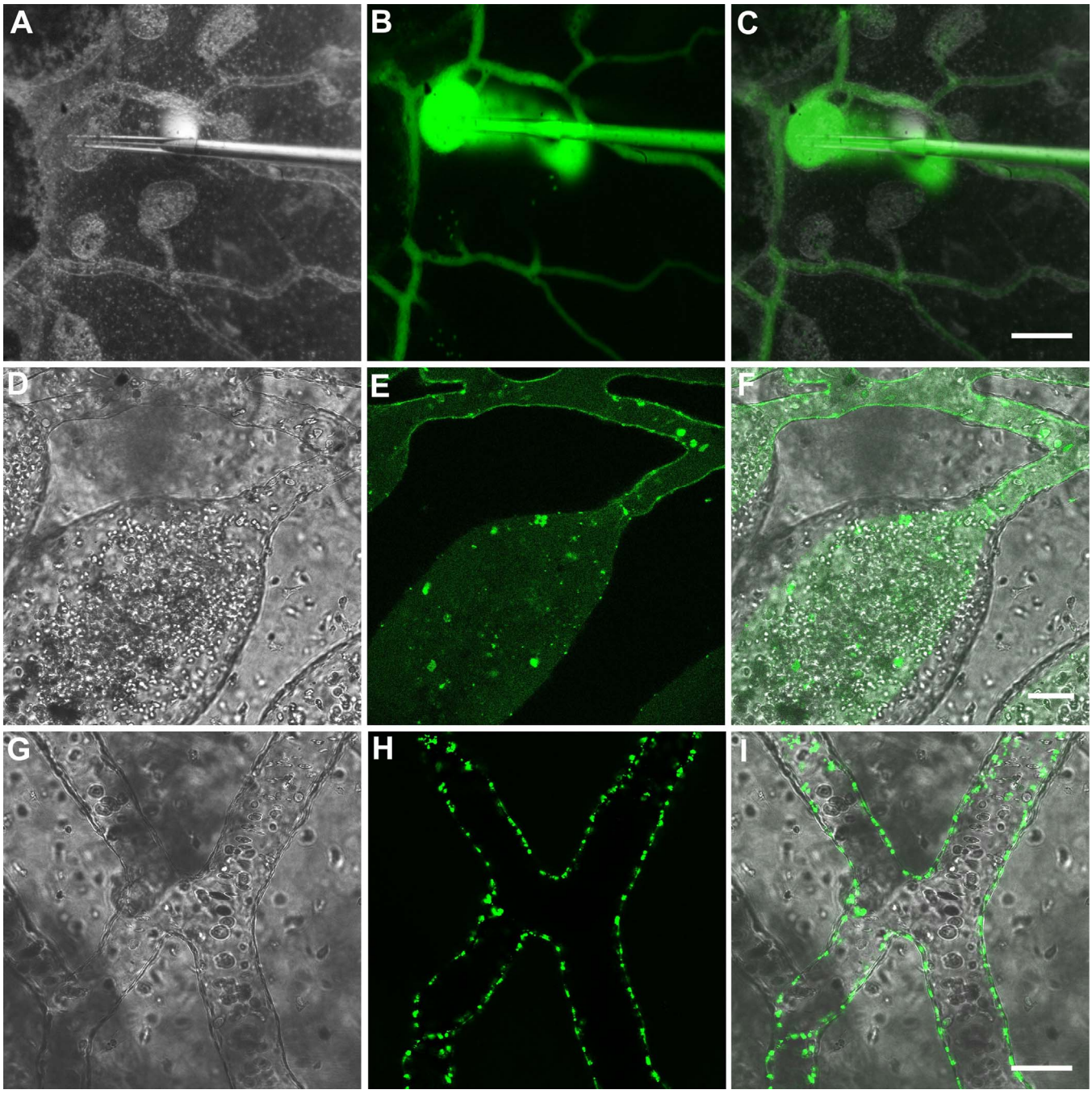

Fig. 5. Microinjections of BSA-488 into the extracorporeal vasculature. A. Bright field image of a microinjection into an ampulla showing the glass needle injecting directly into the blood stream. B. Fluorescent micrograph showing diffusion of BSA-488 and distribution throughout the extracorporeal vasculature. C. Overlay of A and B. D. Bright field image of a microinjected ampulla 10 min after injection. E. Fluorescent micrograph showing the BSA-488 distribution through the extracorporeal vasculature; note that the vascular cells have not up taken the BSA-488. Some auto florescent circulating pigment cells are detected in the blood stream. F. Overlay of D and E. G. Bright field image of blood vessels following 24 h of microinjection. H. Fluorescent micrograph showing the BSA-488 uptake by the cells of the extracorporeal vasculature, note that the absence of circulating BSA-488. Some auto florescent circulating pigment cells are detected in the blood stream. Scale bars A-C 250 μm D-I 50 μm.

and are involved in the allorecognition process between two genetically distinct colonies (Taketa and De Tomaso, 2015). When the ampullae of two compatible colonies come in contact, they can initiate anastomosis and form blood chimeras. In contrast, when colonies are genetically incompatible, the tunic partially fuses and the ampullae leak cells to form a necrotic spot (De Tomaso, 2006; McKitrick and De Tomaso, 2010; Rinkevich, 1996, 2002, 2004; Sabbadin, 1962; Taketa and De Tomaso, 2015). The allorecognition process is dictated by a single locus known as the fusion/histocompatibility (FuHC) locus. Individuals with at least one identical allele are fusion compatible, and those with none are incompatible and trigger rejection (De Tomaso et al., 2005; De Tomaso and Weissman, 2003; Scofield et al., 1982; Weissman et al., 1990). It has been shown that Fester, Uncle Fester, HSP40L, and BHF play a key role during fusion and rejection, and can be used to predict rejection outcomes (De Tomaso et al., 2005; McKitrick et al., 2011; Nydam et al., 2013a, 2013b; Nyholm et al., 2006; Rinkevich et al., 2012; Voskoboynik et al., 2013). Allorecognition proteins are differentially expressed on ampullae compared to blood vessels (McKitrick et al., 2011; Nydam et al., 2013a, 2013b; Nyholm et al., 2006).

Botryllus allorecognition system is highly polymorphic. It has been suggested that its function is to limit vascular anastomosis because blood-based chimeras result in a stem cell competition for the germline

(Laird and De Tomaso, 2005; Laird et al., 2005; Rinkevich and Yankelevich, 2004; Stoner and Weissman, 1996). Ampullae can thus be considered the gatekeepers of invasive parasitic germline stem cells.

Ampullae are distinct tissues when compared to blood vessels. The arrangement of epithelial cells on the leading edge are columnar instead of the simple squamous epithelium that make the blood vessels. Ampullar epithelial cells also express allorecognition genes that are not otherwise expressed in any other tissue (Scofield et al., 1982; Taketa and De Tomaso, 2015). The fusion event is rapid, can happen overnight, and involves remodeling of the tips between fusing ampullae. It has been observed that blood-borne phagocytic cells are present in the fusing ampullae, indicating that they may play a major role during this process (Rinkevich et al., 1998). In the hours following fusion, newly fused ampullae will change their morphology to a blood vessel that is indistinguishable from any other (Braden et al., 2014; Rinkevich et al., 1998; Taketa and De Tomaso, 2015). Vascular and ampullar epithelial cells have different cellular identity, yet they have the plasticity to differentiate into the other cell type, therefore the fusion process is an ideal scenario to study the molecular mechanisms underlying this differentiation process.

7. Anastomosis and parabiosis

Parabiosis comes from Greek words: para meaning alongside, and bios meaning life. It can be defined as the union between two organisms that share a common vasculature and therefore blood supply. Parabiosis in mammals is usually accomplished through surgery, although it can occur naturally during abnormal development, resulting in conjoined individuals (Conboy and Rando, 2012). Experimental parabiosis in mice has lead to major breakthroughs in transplantation, cancer, and rejuvenation of aged organs (Eggel and Wyss-Coray, 2014). Natural occurring parabiosis in *Botryllus* is not age restricted, meaning that it can be achieved between colonies of different ages.

Once fusion of blood vessels between two distinct genotypes occur, they become blood sharing chimeras, and two types of cell competition occur: somatic and germline (Laird and De Tomaso, 2005; Laird et al., 2005; Pancer et al., 1995; Rinkevich, 1996; Rinkevich and Yankelevich, 2004). It has been shown that *Botryllus* colonies have long-lived germline stem cells that recurrently migrate to the newly established niches inside developing buds (Gasparini et al., 2015; Sabbadin and Zaniolo, 1979). A mobile germline is critical in maintaining sexual reproduction in a colony that constantly replaces adult zooids on a weekly basis. Clusters of follicle and germline progenitors become mobile and migrate to niches in both primary and secondary buds during a specific phase of the budding cycle (Langenbacher and De Tomaso, 2016). The migration of germline progenitors to niches is directed by a sphingosine-1-phosphate gradient in the secondary buds (Kassmer et al., 2015). Because of the mobile nature of germline progenitors, fusion between two colonies results in a phenomenon known as germline parasitism, where gametes and progeny of one genotype can be completely replaced by the other (Stoner and Weissman, 1996). The parasitism is persistent through the budding cycles and even after the colonies are disconnected (Stoner et al., 1999). It has been shown that germline stem cells can be prospectively isolated and transplanted directly into the blood stream of a fusible host colony to recapitulate germline parasitism (Laird and De Tomaso, 2005; Laird et al., 2005). This is also true for somatic pigment cell parasitism, although we do understand that cell lineages for somatic and germline are separate.

Parabiosis experiments have been performed between young and old mice to study the effect of young blood in different tissues of the old fusion partner. In these experiments, cardiac hypertrophy in old mice was dramatically reduced and accompanied by decreased cardiomyocyte size after four weeks of parabiosis (Loffredo et al., 2013; Poggioli et al., 2016). GDF11 was identified as a circulating factor in young mice responsible for rescuing cardiac hypertrophy in old mice. Research has shown that aging causes a decline in blood flow leading to a reduction in neural stem cells. Using heterochronic parabiosis caused aged cerebral vasculature to undergo a remodeling, which led to greater blood flow and proliferation of neural stem cells (Katsimpardi et al., 2014).

Young and old vessel fusion may reveal how these differently aged blood vessels and individuals influence each other, and *Botryllus* natural parabiosis is a great model system to study this unique situation.

8. Conclusions and future directions

The extracorporeal vasculature of *Botryllus schlosseri* plays a key role in the life of a colony because it: delivers nutrients to all individuals, regulates the orientation and organization of zooids, dictates the directionality of the expansion and growth of the colony, decides if a fusion or rejection event will happen, serves as a reservoir for germline stem cells, and plays a role in the maturation of developing oocytes. The health of the vasculature is so vital to the survival of a colony that it rapidly regenerates upon injury. In extreme cases when all zooids are removed, it is even able to maintain blood flow and give rise to new zooids (reviewed elsewhere in this issue: Kassmer et al.).

Many aspects related to the remarkable plasticity of the vasculature still remain poorly understood: what signals induce vascular epithelial cells to proliferate or undergo apoptosis? How do the cells interact with the ECM and how do changes in the ECM affect the behavior of the epithelial cells? What signals regulate differentiation into the columnar epithelium of ampullae, and back into blood vessel epithelium?

Because of the accessibility of the BM facing directly to the lumen, *Botryllus* vessels are an ideal model to study mechanotransduction and how epithelial cells respond to chemical or physical stimuli. It has recently been shown that by using small molecule inhibitors it is possible to “soften” the BM, and it would be ideal to study “tightening” the BM using similar approaches.

Long-term effects of parabiosis between age-mismatch colonies and the potential consequences in the physiology of the blood vessels of both parabionts have not been studied in *Botryllus* blood chimeras. We are currently investigating the role of the extracorporeal vasculature and its effects on the senescence of the entire colony.

To better understand the evolution of branching morphogenesis and blood vessel formation it would be ideal to compare different species of colonial ascidians and their ability to respond to either induced regression or expansion of their extracorporeal vasculatures. Earlier comparison have been done, but more modern molecular tools will allow us to understand the specific mechanism behind angiogenesis of invertebrate blood vessels (Mukai and Taneda, 1978). In future studies, analysis of transcriptomics and proteomics of purified populations of vascular cells will identify key genes, proteins, and signaling pathways involved in all different biological aspects of the extracorporeal vasculature.

Acknowledgements

The authors would like to acknowledge Dr. Susannah Kassmer for her input in this review. The authors will also like to acknowledge Claudia Guzik for her micrographs of: BSA-488 immediately after the injections and 24 h later, and the fluorescent in situ hybridization of BsVEGFR. We also acknowledge the use of the NRI-MCDB Microscopy Facility and the Resonant Scanning Confocal supported by NSF MRI Grant 1625770. Finally, the authors would like to acknowledge the grant from the G. Harold and Leila Y. Mathers Foundation (grant ID: SB170066).

References

Alie, A., Hiebert, L.S., Simion, P., Scelzo, M., Prunster, M.M., Lotito, S., Delsuc, F., Douzery, E.J.P., Dantec, C., Lemaire, P., Darras, S., Kawamura, K., Brown, F.D., Tiozzo, S., 2018. Convergent acquisition of nonembryonic development in Styelid Ascidians. Mol. Biol. Evol. 35, 1728–1743.

Anderson, L.H., Boulanger, C.A., Smith, G.H., Carmeliet, P., Watson, C.J., 2011. Stem cell marker prominin-1 regulates branching morphogenesis, but not regenerative capacity, in the mammary gland. Dev. Dyn. 240, 674–681.

Ballarin, L., Menin, A., Tallandini, L., Matozzo, V., Burighel, P., Basso, G., Fortunato, E., Cima, F., 2008. Haemocytes and blastogenetic cycle in the colonial ascidian Botryllus schlosseri: a matter of life and death. Cell Tissue Res. 331, 555–564.

Ballarin, L., Schiavon, F., Manni, L., 2010. Natural apoptosis during the blastogenetic cycle of the colonial ascidian Botryllus schlosseri: a morphological analysis. Zool. Sci. 27, 96–102.

Braden, B.P., Taketa, D.A., Pierce, J.D., Kassmer, S., Lewis, D.D., De Tomaso, A.W., 2014. Vascular regeneration in a basal chordate is due to the presence of immobile, bi-functional cells. PLoS One 9, e95460.

Breier, G., Damert, A., Plate, K.H., Risau, W., 1997. Angiogenesis in embryos and ischemic diseases. Thromb. Haemost. 78, 678–683.

Brown, F.D., Swalla, B.J., 2012. Evolution and development of budding by stem cells: ascidian coloniality as a case study. Dev. Biol. 369, 151–162.

Brunetti, Ra.B., P., 1969. Sviluppo dell'apparato vascolare coloniale in Botryllus schlosseri (Pallas). Pubbl. Staz. Zool. Napoli, 137–148.

Burighel, P., Brunetti, R., 1971. The circulatory system in the blastozooid of the colonial ascidian Botryllus schlosseri (Pallas). Boll. Zool. 38, 273–289.

Campagna, D., Gasparini, F., Franchi, N., Vitulo, N., Ballin, F., Manni, L., Valle, G., Ballarin, L., 2016. Transcriptome dynamics in the asexual cycle of the chordate Botryllus schlosseri. BMC Genom. 17, 275.

Carmeliet, P., Lampugnani, M.G., Moons, L., Breviario, F., Compernolle, V., Bono, F., Balconi, G., Spagnuolo, R., Oosthuyse, B., Dewerchin, M., Zanetti, A., Angellilo, A., Mattot, V., Nuyens, D., Lutgens, E., Clotman, F., de Ruiter, M.C., Gittenberger-de Groot, A., Poelmann, R., Lupu, F., Herbert, J.M., Collen, D., Dejana, E., 1999. Targeted deficiency or cytosolic truncation of the VE-cadherin gene in mice impairs VEGF-mediated endothelial survival and angiogenesis. Cell 98, 147–157.

Chang, W.T., Lauzon, R.J., 1995. Isolation of biologically functional RNA during programmed death of a colonial ascidian. Biol. Bull. 188, 23–31.

Chavez, M.N., Aedo, G., Fierro, F.A., Allende, M.L., Egana, J.T., 2016. Zebrafish as an emerging model organism to study Angiogenesis in development and regeneration. Front. Physiol. 7, 56.

Cima, F., Basso, G., Ballarin, L., 2003. Apoptosis and phosphatidylserine-mediated recognition during the take-over phase of the colonial life-cycle in the ascidian Botryllus schlosseri. Cell Tissue Res. 312, 369–376.

Cima, F., Manni, L., Basso, G., Fortunato, E., Accordi, B., Schiavon, F., Ballarin, L., 2010. Hovering between death and life: natural apoptosis and phagocytes in the blastogenetic cycle of the colonial ascidian Botryllus schlosseri. Dev. Comp. Immunol. 34, 272–285.

Conboy, I.M., Rando, T.A., 2012. Heterochronic parabiosis for the study of the effects of aging on stem cells and their niches. Cell Cycle 11, 2260–2267.

Corbeil, D., Fargeas, C.A., Jaszai, J., 2014. CD133 might be a pan marker of epithelial cells with dedifferentiation capacity. Proc. Natl. Acad. Sci. USA 111, E1451–E1452.

Craig, M.P., Sumanas, S., 2016. ETS transcription factors in embryonic vascular development. Angiogenesis 19, 275–285.

Davies, J.A., 2002. Do different branching epithelia use a conserved developmental mechanism? Bioessays 24, 937–948.

Davies, J.A., 2005. Watching tubules glow and branch. Curr. Opin. Genet. Dev. 15, 364–370.

De Spiegelaere, W., Casteleyn, C., Van den Broeck, W., Plendl, J., Bahramsoltani, M., Simoens, P., Djonov, V., Cornillie, P., 2012. Intussusceptive angiogenesis: a biologically relevant form of angiogenesis. J. Vasc. Res. 49, 390–404.

De Tomaso, A.W., 2006. Allorecognition polymorphism versus parasitic stem cells. Trends Genet. 22, 485–490.

De Tomaso, A.W., Nyholm, S.V., Palmeri, K.J., Ishizuka, K.J., Ludington, W.B., Mitchel, K., Weissman, I.L., 2005. Isolation and characterization of a protochordate histocompatibility locus. Nature 438, 454–459.

De Tomaso, A.W., Weissman, I.L., 2003. Initial characterization of a protochordate histocompatibility locus. Immunogenetics 55, 480–490.

Delsuc, F., Brinkmann, H., Chourrout, D., Philippe, H., 2006. Tunicates and not cephalochordates are the closest living relatives of vertebrates. Nature 439, 965–968.

Delsuc, F., Philippe, H., Tsagkogeorga, G., Simion, P., Tilak, M.K., Turon, X., Lopez-Legentil, S., Piette, J., Lemaire, P., Douzery, E.J.P., 2018. A phylogenomic framework and timescale for comparative studies of tunicates. BMC Biol. 16, 39.

DiPietro, L.A., 2016. Angiogenesis and wound repair: when enough is enough. J. Leukoc. Biol. 100, 979–984.

Eggel, A., Wyss-Coray, T., 2014. A revival of parabiosis in biomedical research. Swiss Med. Wkly. 144, w13914.

Flamme, I., Frolich, T., Risau, W., 1997. Molecular mechanisms of vasculogenesis and embryonic angiogenesis. J. Cell Physiol. 173, 206–210.

Folberg, R., Maniotis, A.J., 2004. Vasculogenic mimicry. APMIS 112, 508–525.

Franchi, N., Ballin, F., Manni, L., Schiavon, F., Ballarin, L., 2016a. Data on four apoptosis-related genes in the colonial tunicate Botryllus schlosseri. Data Brief 8, 142–152.

Franchi, N., Ballin, F., Manni, L., Schiavon, F., Basso, G., Ballarin, L., 2016b. Recurrent phagocytosis-induced apoptosis in the cyclical generation change of the compound ascidian Botryllus schlosseri. Dev. Comp. Immunol. 62, 8–16.

Fruttiger, M., 2002. Development of the mouse retinal vasculature: angiogenesis versus vasculogenesis. Investig. Ophthalmol. Vis. Sci. 43, 522–527.

Gariano, R.F., 2003. Cellular mechanisms in retinal vascular development. Prog. Retin Eye Res. 22, 295–306.

Gasparini, F., Burighel, P., Manni, L., Zaniolo, G., 2008. Vascular regeneration and angiogenic-like sprouting mechanism in a compound ascidian is similar to vertebrates. Evol. Dev. 10, 591–605.

Gasparini, F., Caicci, F., Rigon, F., Zaniolo, G., Manni, L., 2014. Testing an unusual in vivo vessel network model: a method to study angiogenesis in the colonial tunicate Botryllus schlosseri. Sci. Rep. 4, 6460.

Gasparini, F., Longo, F., Manni, L., Burighel, P., Zaniolo, G., 2007. Tubular sprouting as a mode of vascular formation in a colonial ascidian (Tunicata). Dev. Dyn. 236, 719–731.

Gasparini, F., Manni, L., Cima, F., Zaniolo, G., Burighel, P., Caicci, F., Franchi, N., Schiavon, F., Rigon, F., Campagna, D., Ballarin, L., 2015. Sexual and asexual reproduction in the colonial ascidian Botryllus schlosseri. Genesis 53, 105–120.

Gerhardt, H., Betsholtz, C., 2005. How do endothelial cells orientate? EXS, 3–15.

Gory-Faure, S., Prandini, M.H., Pointu, H., Roullot, V., Pignot-Paintrand, I., Vernet, M., Huber, P., 1999. Role of vascular endothelial-cadherin in vascular morphogenesis. Development 126, 2093–2102.

Guzman, A., Macias-Valencia, R., Fierro-Fierro, F., Gutierrez, C.G., Rosales-Torres, A.M., 2015. The corpora lutea proangiogenic state of VEGF system components is turned to antiangiogenic at the later phase of the oestrous cycle in cows. Animal 9, 301–307.

Hickey, M., Fraser, I., 2003. Human uterine vascular structures in normal and diseased states. Microsc. Res. Tech. 60, 377–389.

Holland, L.Z., 2016. Tunicates. Curr. Biol. 26, R146–R152.

Jang, J.W., Song, Y., Kim, S.H., Kim, J., Seo, H.R., 2017. Potential mechanisms of CD133 in cancer stem cells. Life Sci. 184, 25–29.

Kalluri, R., 2003. Basement membranes: structure, assembly and role in tumour angiogenesis. Nat. Rev. Cancer 3, 422–433.

Karbanova, J., Laco, J., Marzesco, A.M., Janich, P., Vobornikova, M., Mokry, J., Fargeas, C.A., Huttner, W.B., Corbeil, D., 2014. Human prominin-1 (CD133) is detected in both neoplastic and non-neoplastic salivary gland diseases and released into saliva in a ubiquitinated form. PLoS One 9, e98927.

Kassmer, S.H., Rodriguez, D., Langenbacher, A.D., Bui, C., De Tomaso, A.W., 2015. Migration of germline progenitor cells is directed by sphingosine-1-phosphate signalling in a basal chordate. Nat. Commun. 6, 8565.

Katsimpardi, L., Litterman, N.K., Schein, P.A., Miller, C.M., Loffredo, F.S., Wojtkiewicz, G.R., Chen, J.W., Lee, R.T., Wagers, A.J., Rubin, L.L., 2014. Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors. Science 344, 630–634.

Kocot, K.M., Tassia, M.G., Halanych, K.M., Swalla, B.J., 2018. Phylogenomics offers resolution of major tunicate relationships. Mol. Phylogenet. Evol. 121, 166–173.

Korn, C., Augustin, H.G., 2015. Mechanisms of vessel pruning and regression. Dev. Cell 34, 5–17.

Kramann, R., Kusaba, T., Humphreys, B.D., 2015. Who regenerates the kidney tubule? Nephrol. Dial. Transplant. 30, 903–910.

Kucera, T., Lammert, E., 2009. Ancestral vascular tube formation and its adoption by tumors. Biol. Chem. 390, 985–994.

Kusaba, T., Lalli, M., Kramann, R., Kobayashi, A., Humphreys, B.D., 2014. Differentiated kidney epithelial cells repair injured proximal tubule. Proc. Natl. Acad. Sci. USA 111, 1527–1532.

Laird, D.J., De Tomaso, A.W., 2005. Predatory stem cells in the non-zebrafish chordate, Botryllus schlosseri. Zebrafish 1, 357–361.

Laird, D.J., De Tomaso, A.W., Weissman, I.L., 2005. Stem cells are units of natural selection in a colonial ascidian. Cell 123, 1351–1360.

Langenbacher, A.D., De Tomaso, A.W., 2016. Temporally and spatially dynamic germ cell niches in Botryllus schlosseri revealed by expression of a TGF-beta family ligand and vasa. Evodevo 7, 9.

Lauzon, R.J., Ishizuka, K.J., Weissman, I.L., 1992. A cyclical, developmentally-regulated death phenomenon in a colonial urochordate. Dev. Dyn. 194, 71–83.

Lauzon, R.J., Ishizuka, K.J., Weissman, I.L., 2002. Cyclical generation and degeneration of organs in a colonial urochordate involves crosstalk between old and new: a model for development and regeneration. Dev. Biol. 249, 333–348.

Lauzon, R.J., Kidder, S.J., Long, P., 2007. Suppression of programmed cell death regulates the cyclical degeneration of organs in a colonial urochordate. Dev. Biol. 301, 92–105.

Lauzon, R.J., Patton, C.W., Weissman, I.L., 1993. A morphological and immunohistochemical study of programmed cell death in Botryllus schlosseri (Tunicata, Ascidiacea). Cell Tissue Res. 272, 115–127.

Lof-Ohlin, Z.M., Nyeng, P., Bechard, M.E., Hess, K., Bankaitis, E., Greiner, T.U., Ameri, J., Wright, C.V., Semb, H., 2017. EGFR signalling controls cellular fate and pancreatic organogenesis by regulating apicobasal polarity. Nat. Cell Biol. 19, 1313–1325.

Loffredo, F.S., Steinhauser, M.L., Jay, S.M., Gannon, J., Pancoast, J.R., Yalamanchi, P., Sinha, M., Dall'Osso, C., Khong, D., Shadrach, J.L., Miller, C.M., Singer, B.S., Stewart, A., Psychogios, N., Gerszten, R.E., Hartigan, A.J., Kim, M.J., Serwold, T., Wagers, A.J., Lee, R.T., 2013. Growth differentiation factor 11 is a circulating factor that reverses age-related cardiac hypertrophy. Cell 153, 828–839.

Lubarsky, B., Krasnow, M.A., 2003. Tube morphogenesis: making and shaping biological tubes. Cell 112, 19–28.

Makanya, A.N., Hlushchuk, R., Djonov, V.G., 2009. Intussusceptive angiogenesis and its role in vascular morphogenesis, patterning, and remodeling. Angiogenesis 12, 113–123.

Matthysse, A.G., Deschet, K., Williams, M., Marry, M., White, A.R., Smith, W.C., 2004. A functional cellulose synthase from ascidian epidermis. Proc. Natl. Acad. Sci. USA

101, 986–991.
McKitrick, T.R., De Tomaso, A.W., 2010. Molecular mechanisms of allorecognition in a basal chordate. Semin. Immunol. 22, 34–38.
McKitrick, T.R., Muscat, C.C., Pierce, J.D., Bhattacharya, D., De Tomaso, A.W., 2011. Allorecognition in a basal chordate consists of independent activating and inhibitory pathways. Immunity 34, 616–626.
McNally, S., Stein, T., 2017. Overview of mammary gland development: a comparison of mouse and human. Methods Mol. Biol. 1501, 1–17.
Modlich, U., Kaup, F.J., Augustin, H.G., 1996. Cyclic angiogenesis and blood vessel regression in the ovary: blood vessel regression during luteolysis involves endothelial cell detachment and vessel occlusion. Lab. Investig. 74, 771–780.
Mukai, H.S., Sugimoto, K., Taneda, Y., 1978. Comparative studies on the circulatory system of the compound ascidians: botryllus, Botrylloides and Symplegma. J. Morphol., 49–78.
Munoz-Chapuli, R., 2011. Evolution of angiogenesis. Int. J. Dev. Biol. 55, 345–351.
Munoz-Chapuli, R., Carmona, R., Guadix, J.A., Macias, D., Perez-Pomares, J.M., 2005. The origin of the endothelial cells: an evo-devo approach for the invertebrate/vertebrate transition of the circulatory system. Evol. Dev. 7, 351–358.
Nydam, M.L., Hoang, T.A., Shanley, K.M., De Tomaso, A.W., 2013a. Molecular evolution of a polymorphic HSP40-like protein encoded in the histocompatibility locus of an invertebrate chordate. Dev. Comp. Immunol. 41, 128–136.
Nydam, M.L., Netuschil, N., Sanders, E., Langenbacher, A., Lewis, D.D., Taketa, D.A., Marimuthu, A., Gracey, A.Y., De Tomaso, A.W., 2013b. The candidate histocompatibility locus of a Basal chordate encodes two highly polymorphic proteins. PLoS One 8, e65980.
Nyholm, S.V., Passegue, E., Ludington, W.B., Voskoboynik, A., Mitchel, K., Weissman, I.L., De Tomaso, A.W., 2006. fester, A candidate allorecognition receptor from a primitive chordate. Immunity 25, 163–173.
O'Brien, J., Lyons, T., Monks, J., Lucia, M.S., Wilson, R.S., Hines, L., Man, Y.G., Borges, V., Schedin, P., 2010. Alternatively activated macrophages and collagen remodeling characterize the postpartum involuting mammary gland across species. Am. J. Pathol. 176, 1241–1255.
Onat, D., Brillon, D., Colombo, P.C., Schmidt, A.M., 2011. Human vascular endothelial cells: a model system for studying vascular inflammation in diabetes and atherosclerosis. Curr. Diab. Rep. 11, 193–202.
Pancer, Z., Gershon, H., Rinkevich, B., 1995. Coexistence and possible parasitism of somatic and germ cell lines in chimeras of the colonial urochordate Botryllus schlosseri. Biol. Bull. 189, 106–112.
Patricolo, E., Ferrarella, A., 1973. Histochemical and biochemical studies on the collagen of the tunica of Ciona intestinalis. Riv. Biol. 66, 115–134.
Paulis, Y.W., Soetekouw, P.M., Verheul, H.M., Tjan-Heijnen, V.C., Griffioen, A.W., 2010. Signalling pathways in vasculogenic mimicry. Biochim. Biophys. Acta 1806, 18–28.
Poggioli, T., Vujic, A., Yang, P., Macias-Trevino, C., Uygur, A., Loffredo, F.S., Pancoast, J.R., Cho, M., Goldstein, J., Tandias, R.M., Gonzalez, E., Walker, R.G., Thompson, T.B., Wagers, A.J., Fong, Y.W., Lee, R.T., 2016. Circulating growth differentiation factor 11/8 levels decline with age. Circ. Res. 118, 29–37.
Ribatti, D., 2009. The discovery of antiangiogenic molecules: a historical review. Curr. Pharm. Des. 15, 345–352.
Ricciuti, B., Foglietta, J., Bianconi, V., Sahebkar, A., Pirro, M., 2017a. Enzymes involved in tumor-driven angiogenesis: a valuable target for anticancer therapy. Semin. Cancer Biol..
Ricciuti, B., Foglietta, J., Chiari, R., Sahebkar, A., Banach, M., Bianconi, V., Pirro, M., 2017b. Emerging enzymatic targets controlling angiogenesis in cancer: preclinical evidence and potential clinical applications. Med. Oncol. 35, 4.
Rinkevich, B., 1996. Bi-versus multichimerism in colonial urochordates: a hypothesis for links between natural tissue transplantation, allogenetics and evolutionary ecology. Exp. Clin. Immunogenet. 13, 61–69.
Rinkevich, B., 2002. The colonial urochordate Botryllus schlosseri: from stem cells and natural tissue transplantation to issues in evolutionary ecology. Bioessays 24, 730–740.
Rinkevich, B., 2004. Primitive immune systems: are your ways my ways? Immunol. Rev. 198, 25–35.
Rinkevich, B., Douek, J., Rabinowitz, C., Paz, G., 2012. The candidate Fu/HC gene in Botryllusschlosseri (Urochordata) and ascidians' historecognition–an oxymoron? Dev. Comp. Immunol. 36, 718–727.
Rinkevich, B., Lauzon, R.J., Brown, B.W., Weissman, I.L., 1992. Evidence for a programmed life span in a colonial protochordate. Proc. Natl. Acad. Sci. USA 89, 3546–3550.
Rinkevich, B., Weissman, I.L., De Tomaso, A.W., 1998. Transplantation of Fu/HC-incompatible zooids in Botryllus schlosseri results in chimerism. Biol. Bull. 195, 98–106.
Rinkevich, B., Yankelevich, I., 2004. Environmental split between germ cell parasitism and somatic cell synergism in chimeras of a colonial urochordate. J. Exp. Biol. 207, 3531–3536.
Risau, W., 1997. Mechanisms of angiogenesis. Nature 386, 671–674.
Risau, W., Flamme, I., 1995. Vasculogenesis. Annu. Rev. Cell Dev. Biol. 11, 73–91.
Risau, W., Sariola, H., Zerwes, H.G., Sasse, J., Ekblom, P., Kemler, R., Doetschman, T., 1988. Vasculogenesis and angiogenesis in embryonic-stem-cell-derived embryoid bodies. Development 102, 471–478.
Rocha, L.A., Learmonth, D.A., Sousa, R.A., Salgado, A.J., 2018. alphavbeta3 and alpha5beta1 integrin-specific ligands: from tumor angiogenesis inhibitors to vascularization promoters in regenerative medicine? Biotechnol. Adv. 36, 208–227.
Rodriguez, C., Martinez-Gonzalez, J., Raposo, B., Alcudia, J.F., Guadall, A., Badimon, L., 2008. Regulation of lysyl oxidase in vascular cells: lysyl oxidase as a new player in cardiovascular diseases. Cardiovasc. Res. 79, 7–13.
Rodriguez, D., Braden, B.P., Boyer, S.W., Taketa, D.A., Setar, L., Calhoun, C., Maio, A.D., Langenbacher, A., Valentine, M.T., De Tomaso, A.W., 2017a. In vivo manipulation of the extracellular matrix induces vascular regression in a basal chordate. Mol. Biol. Cell 28, 1883–1893.
Rodriguez, D., Kassmer, S.H., De Tomaso, A.W., 2017b. Gonad development and hermaphroditism in the ascidian Botryllus schlosseri. Mol. Reprod. Dev. 84, 158–170.
Rodriguez, D., Sanders, E.N., Farell, K., Langenbacher, A.D., Taketa, D.A., Hopper, M.R., Kennedy, M., Gracey, A., De Tomaso, A.W., 2014. Analysis of the basal chordate Botryllus schlosseri reveals a set of genes associated with fertility. BMC Genom. 15, 1183.
Rosengarten, R.D., Nicotra, M.L., 2011. Model systems of invertebrate allorecognition. Curr. Biol. 21, R82–R92.
Rosner, A., Rabinowitz, C., Moiseeva, E., Voskoboynik, A., Rinkevich, B., 2007. BS-cadherin in the colonial urochordate Botryllus schlosseri: one protein, many functions. Dev. Biol. 304, 687–700.
Sabbadin, A., 1962. Le basi genetiche della capacità di fusione fra colonie in Botryllus schlosseri (Ascidiacea). Rend. Accad. Naz. Lincei (Cl. Sci. FF MM NN) 32, 1031–1035.
Sabbadin, A., Zaniolo, G., 1979. Sexual differentiation and germ-cell transfer in the colonial ascidian Botryllus-Schlosseri. J. Exp. Zool. 207, 289–304.
Schittny, J.C., 2017. Development of the lung. Cell Tissue Res. 367, 427–444.
Scofield, V.L., Schlumpberger, J.M., Weissman, I.L., 1982. Colony specificity in the colonial tunicate Botryllus and the origins of vertebrate immunity. Am. Zool. 22, 783–794.
Shih, H.P., Wang, A., Sander, M., 2013. Pancreas organogenesis: from lineage determination to morphogenesis. Annu. Rev. Cell Dev. Biol. 29, 81–105.
Shoda, T., Futamura, K., Orihara, K., Emi-Sugie, M., Saito, H., Matsumoto, K., Matsuda, A., 2016. Recent advances in understanding the roles of vascular endothelial cells in allergic inflammation. Allergol. Int. 65, 21–29.
Silanikove, N., 2014. Natural and abrupt involution of the mammary gland affects differently the metabolic and health consequences of weaning. Life Sci. 102, 10–15.
Stoner, D.S., Rinkevich, B., Weissman, I.L., 1999. Heritable germ and somatic cell lineage competitions in chimeric colonial protochordates. Proc. Natl. Acad. Sci. USA 96, 9148–9153.
Stoner, D.S., Weissman, I.L., 1996. Somatic and germ cell parasitism in a colonial ascidian: possible role for a highly polymorphic allorecognition system. Proc. Natl. Acad. Sci. USA 93, 15254–15259.
Strazzabosco, M., Fabris, L., 2012. Development of the bile ducts: essentials for the clinical hepatologist. J. Hepatol. 56, 1159–1170.
Strilic, B., Kucera, T., Lammert, E., 2010. Formation of cardiovascular tubes in invertebrates and vertebrates. Cell Mol. Life Sci. 67, 3209–3218.
Sweat, R.S., Sloas, D.C., Stewart, S.A., Czarny-Ratajczak, M., Baddoo, M., Eastwood, J.R., Suarez-Martinez, A.D., Azimi, M.S., Burks, H.E., Chedister, L.O., Myers, L., Murfee, W.L., 2017. Aging is associated with impaired angiogenesis, but normal microvascular network structure, in the rat mesentery. Am. J. Physiol. Heart Circ. Physiol. 312, H275–H284.
Taddei, M.L., Giannoni, E., Fiaschi, T., Chiarugi, P., 2012. Anoikis: an emerging hallmark in health and diseases. J. Pathol. 226, 380–393.
Taketa, D.A., De Tomaso, A.W., 2015. Botryllus schlosseri allorecognition: tackling the enigma. Dev. Comp. Immunol. 48, 254–265.
Tiozzo, S., Ballarin, L., Burighel, P., Zaniolo, G., 2006. Programmed cell death in vegetative development: apoptosis during the colonial life cycle of the ascidian Botryllus schlosseri. Tissue Cell 38, 193–201.
Tiozzo, S., Voskoboynik, A., Brown, F.D., De Tomaso, A.W., 2008. A conserved role of the VEGF pathway in angiogenesis of an ectodermally-derived vasculature. Dev. Biol. 315, 243–255.
Treps, L., Gavard, J., 2015. Tumor angiogenesis: when the tree of life turns bad. Med. Sci. 31, 989–995.
Vailhe, B., Vittet, D., Feige, J.J., 2001. In vitro models of vasculogenesis and angiogenesis. Lab. Investig. 81, 439–452.
van der Bilt, J.D., Borel Rinkes, I.H., 2004. Surgery and angiogenesis. Biochim. Biophys. Acta 1654, 95–104.
Voskoboynik, A., Newman, A.M., Corey, D.M., Sahoo, D., Pushkarev, D., Neff, N.F., Passarelli, B., Koh, W., Ishizuka, K.J., Palmeri, K.J., Dimov, I.K., Keasar, C., Fan, H.C., Mantalas, G.L., Sinha, R., Penland, L., Quake, S.R., Weissman, I.L., 2013. Identification of a colonial chordate histocompatibility gene. Science 341, 384–387.
Voskoboynik, A., Rinkevich, B., Weiss, A., Moiseeva, E., Reznick, A.Z., 2004. Macrophage involvement for successful degeneration of apoptotic organs in the colonial urochordate Botryllus schlosseri. J. Exp. Biol. 207, 2409–2416.
Wallez, Y., Vilgrain, I., Huber, P., 2006. Angiogenesis: the VE-cadherin switch. Trends Cardiovasc. Med. 16, 55–59.
Walls, J.R., Coultas, L., Rossant, J., Henkelman, R.M., 2008. Three-dimensional analysis of vascular development in the mouse embryo. PLoS One 3, e2853.
Wang, D., 2008. Discrepancy between mRNA and protein abundance: insight from information retrieval process in computers. Comput. Biol. Chem. 32, 462–468.
Watson, E.C., Grant, Z.L., Coultas, L., 2017. Endothelial cell apoptosis in angiogenesis and vessel regression. Cell Mol. Life Sci. 74, 4387–4403.
Wei, J., Wang, G., Li, X., Ren, P., Yu, H., Dong, B., 2017. Architectural delineation and molecular identification of extracellular matrix in ascidian embryos and larvae. Biol. Open 6, 1383–1390.
Weinstein, B.M., 2005. Vessels and nerves: marching to the same tune. Cell 120, 299–302.
Weissman, I.L., Saito, Y., Rinkevich, B., 1990. Allorecognition histocompatibility in a protochordate species: is the relationship to MHC somatic or structural? Immunol. Rev. 113, 227–241.
Xu, C.S., Zhang, S.B., Chen, X.G., Rahman, S., 2007. Correlation analysis of liver tumor-

associated genes with liver regeneration. World J. Gastroenterol. 13, 3323–3332.
Zacchigna, S., de Almodovar, Ruiz, Carmeliet, P, C., 2008. Similarities between angiogenesis and neural development: what small animal models can tell us. Curr. Top. Dev. Biol. 80, 1–55.
Zaniolo, G., 1981. Histology of the ascidian Botryllus schlosseri tunic: in particular, the test cells. Boll. Zool., 169–178.
Zaniolo, G., Trentin, P., 1987. Regeneration of the tunic in the colonial ascidian, Botryllus schlosseri. Acta Embryol. Morphol. Exp., 173–180.
Zarzynska, J., Motyl, T., 2008. Apoptosis and autophagy in involuting bovine mammary gland. J. Physiol. Pharmacol. 59 (Suppl. 9), S275–S288.
Zecchin, A., Kalucka, J., Dubois, C., Carmeliet, P., 2017. How endothelial cells adapt their metabolism to form vessels in tumors. Front. Immunol. 8, 1750.