

Review Article

Kindlin-3 in the immune system

Susanna C Fagerholm^{1,2}, Hwee San Lek², Vicky L Morrison²

¹Institute of Biotechnology, University of Helsinki, Finland; ²Medical Research Institute, Ninewells Hospital and Medical School, University of Dundee, UK

Received October 16, 2013; Accepted December 12, 2013; Epub February 27, 2014; Published March 15, 2014

Abstract: Kindlin-3 is a member of the kindlin family of focal adhesion proteins which bind to integrin beta-chain cytoplasmic domains to regulate integrin function. In contrast to kindlin-1 and kindlin-2 proteins, kindlin-3 is expressed mainly in the hematopoietic system. Mutations in kindlin-3 result in the rare genetic disorder, leukocyte adhesion deficiency type III (LAD-III), which is characterized by bleeding and recurrent infections due to deficient beta1, beta2 and beta3 integrin activation in platelets and leukocytes. Here, we review the role of kindlin-3 in integrin activation and in different immune cell functions.

Keywords: Kindlin, integrin, adhesion, LAD-III, T cell

Introduction

Integrins of the beta2-family (alphaLbeta2; CD11a/CD18; LFA-1, alphaMbeta2; CD11b/CD18; Mac-1, alphaXbeta2; CD11c/CD18 and alphaDbeta2; CD11d/CD18 integrins) play a vital role in leukocyte adhesion and migration *in vivo*. These receptors are exclusively expressed on leukocytes and bind to ligands such as intercellular adhesion molecules (ICAMs) on other leukocytes and endothelial cells, as well as soluble proteins such as the complement protein iC3b. beta2-integrins are essential for the firm adhesion of leukocytes to endothelial cells in the blood vessels during shear flow conditions [1]. They have also been reported to be important for neutrophil activation, T cell-dendritic cell contact formation and T cell killing of target cells. In addition, alphaM-beta2 and alphaXbeta2-integrins are important receptors for complement (iC3b) and mediate phagocytosis of complement-coated particles. A low or absent expression of beta2-integrins causes leukocyte adhesion deficiency type I (LAD-I), a rare genetic condition where leukocytes (especially neutrophils) cannot exit the blood stream, resulting in recurrent bacterial infections and fungal infections and delayed wound healing, showing the crucial importance of these receptors in immunity [2].

Leukocytes also express other integrins, such as alpha4beta1-integrin (VLA-4), which plays important roles in lymphocyte trafficking through binding to ligands such as vascular cell adhesion molecule-1 (VCAM-1).

The alphallbbeta3 integrin is expressed on platelets and binds to ligands such as fibronectin, fibrinogen, von Willebrandt factor and thrombospondin. This integrin is important for platelet aggregation, and deficiencies in the function of this receptor are associated with Glanzmann thrombasthenia, a bleeding disorder [3].

Kindlin-3 binds integrin beta-chain cytoplasmic domains and regulates integrin function

Integrins are regulated by the binding of cytoplasmic factors, such as talin, filamin, 14-3-3 proteins and kindlin to their intracellular tails, and these interactions are of crucial importance for integrin function [1, 4, 5].

The kindlin family of focal adhesion proteins consists of three members. Kindlin-1 is expressed mainly in epithelial cells and keratinocytes, and mutations in kindlin-1 lead to skin blistering, skin atrophy and periodontal disease in patients with Kindler Syndrome [6]. Kindlin-2 is ubiquitously expressed and kindlin-2 knock-

Kindlin-3 in immunity

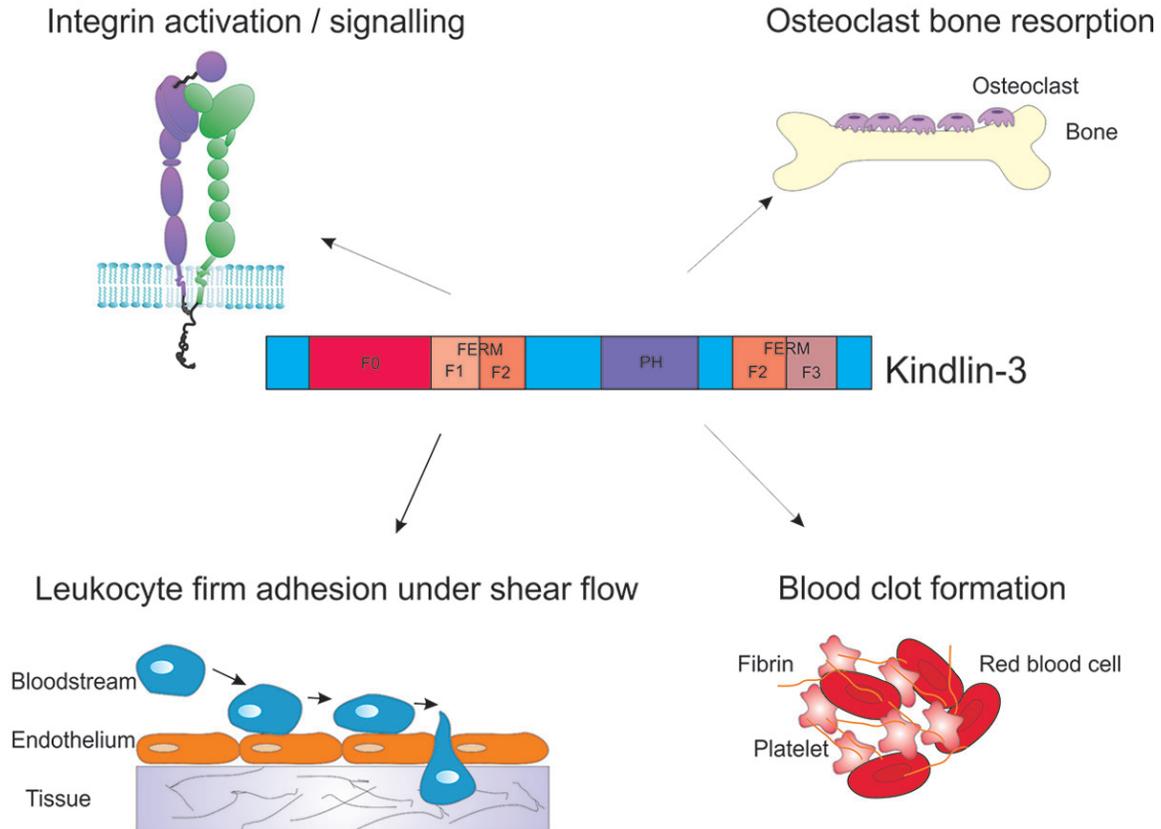


Figure 1. Schematic diagram of kindlin-3 functions in cells. The integrin binding site is in the F3 subdomain of kindlin-3.

out mice are embryonic lethal at the preimplantation stage [7]. Kindlin-2 is necessary for myogenesis and myocyte elongation [8, 9], and mice with kindlin-2 deficiency (kindlin-2 +/- animals) display defective angiogenesis and a leaky and immature vasculature [10].

In contrast to kindlin-1 and kindlin-2, kindlin-3 is expressed mainly in hematopoietic cells [11], although expression in endothelial cells has also been described [12]. Kindlin-3 contains a FERM domain and a PH-domain and adopts an elongated conformation which is similar to that of talin's head domain [13] (**Figure 1**).

Recently, it was discovered that kindlin-3 is mutated in the rare genetic disorder, leukocyte adhesion deficiency type III (LAD-III). Like LAD-I patients, LAD-III patients suffer from recurrent infections, but in addition, the patients have a bleeding phenotype due to deficient platelet integrin activation [14-16]. In addition, the patients may suffer from osteopetrosis [15], due to deficient integrin-mediated osteoclast

bone resorption [17]. Kindlin-3 binds to beta1, beta2 and beta3 integrin cytoplasmic domains and regulates integrin functions *in vivo*. The kindlin binding site in the integrin beta2-chain consists of the membrane-distal NPXY-motif [5] and a TTT-domain in the beta2-integrin tail [13, 18]. The kindlin binding site does not overlap with the binding site for talin, another important integrin regulator which binds to the membrane-proximal NPXY-motif [5, 18]. However, the kindlin-3 binding site does overlap with binding sites for other integrin interactors, eg filamin, a negative regulator of integrins [4] and 14-3-3 proteins, which bind to phosphorylated beta2-integrins and appears to regulate Rac-1 signalling and cell spreading downstream of the integrin [4, 19, 20]. In addition, sorting nexin 17, a protein which regulates integrin recycling has been reported to bind to a similar site in beta1-integrins [21]. Whether there is competition between these proteins for the binding site in beta2-integrins, and what the functional consequences of such competition would be is unknown at present.

Kindlin-3 in immunity

Table 1. Comparison of talin and kindlin-3 functions in integrin regulation and the immune system

| | Talin -/- | Kindlin-3 -/- | References |
|------------------------------------|--------------------------------|---|-------------|
| Integrin conformation | Low affinity conformation only | Low and intermediate affinity conformations | [27] |
| Integrin outside-in signalling | Impaired | Impaired | [17] |
| Lymphocyte development | ? (embryonic lethal) | Impaired | [5, 35] |
| Leukocyte rolling under shear flow | Impaired | Normal | [18, 27] |
| Leukocyte firm adhesion/spreading | Impaired | Impaired | [5, 18, 28] |
| T cell activation (spleen) | Impaired | Normal | [18, 28] |

Kindlin-3 knockout animals die early after birth due to severe bleeding because of a deficiency in α IIb β 3 integrin activation and platelet aggregation [22]. Study of kindlin-3 deficient neutrophils have revealed an essential role for kindlin-3 in the regulation of neutrophil adhesion to endothelial cells under shear flow *in vivo* [5], and studies of other (human and mouse) immune cell types has revealed a similar role for kindlin-3 in other leukocytes, such as B cells [23], CD4 T cells and effector T cells [18, 24, 25] and NK cells [26] (**Figure 1**).

Because kindlin-3 knockout animals die early after birth due to the bleeding phenotype, and because lymphocyte development is affected in these animals [5], it has remained unclear how kindlin-3 regulates β 2-integrins in lymphocytes *in vivo*. The development of a novel animal model, where the kindlin-3 binding site in the β 2-integrin has been mutated (TTT/AAA- β 2-integrin knock-in mouse model), has confirmed the importance of kindlin-3 in regulation of leukocyte adhesion *in vivo*, as these animals display splenomegaly and neutrophilia [18]. Lymphocyte development is normal in these mice, but kindlin-3 binding to the β 2-integrin cytoplasmic domain is essential for chemokine-induced CD4+ T cell adhesion to ICAM-1 and endothelial cells under shear flow conditions and for normal lymphocyte recirculation and homing to lymph nodes. In addition, the integrin-kindlin interaction is necessary for effector T cell adhesion under flow conditions [18].

Kindlin-3 is not necessary for all integrin-mediated functions in the immune system

Clues to how kindlin-3 regulates integrins have come from detailed investigations of integrin function in kindlin-3 deficient cells or cells where the interaction between kindlin-3 and the integrin has been abolished. Neutrophils deficient in talin are deficient in both rolling and arrest, whilst kindlin deficient neutrophils can

still mediate slow rolling [27], indicating that these different integrin regulators serve different roles in integrin activation (**Table 1**). Neither talin nor kindlin-3 deficient neutrophils were able to adopt the high-affinity conformation (extended/open I domain) of the LFA-1 integrin, whilst only talin -/- cells were deficient the first step of integrin activation to the intermediate affinity form (extended/closed I domain) [27].

We have recently shown that the integrin/kindlin interaction is not completely necessary for integrin-mediated effector T cell 2-dimensional migration on ICAM-1, or for β 2-integrin mediated rolling on ICAM-1 under shear flow conditions [18]. Atomic force microscopy measurements of early interactions between the integrin and its ligand have revealed that such early interactions in the absence of shear flow is normal [18]. Probably because of this, integrin-kindlin interactions is not essential for T cell activation in the spleen *in vivo*, which occurs in an environment without shear stress [18]. In contrast, talin is necessary for T cell activation *in vivo* [28]. In addition, recent studies have shown that kindlin-3 is not necessary for effector T cell diapedesis [25]. Interestingly, it is also not necessary for the induction of experimental autoimmune encephalomyelitis in mice, although it is necessary for recruitment of autoreactive effector T cells into the naïve CNS [24]. These different requirements for kindlin-3 in integrin regulation in effector T cells appear to reflect differences in ligand levels on resting and inflamed endothelium; kindlin-3 appears to be required only for adhesion to low, but not high, levels of integrin ligands ICAM-1 and VCAM-1. Collectively, these studies reveal the selective role of the integrin/kindlin interactions in regulating integrin-mediated adhesive functions (**Table 1**).

Outside-in signaling

In addition to its role in integrin activation to mediate adhesion under shear flow conditions,

Kindlin-3 is thought to be involved in integrin outside-in signaling [29]. Integrin-mediated cell spreading is deficient in kindlin-3 $-/-$ neutrophils [5] and in effector T cells where the kindlin-3 binding site is mutated in the beta2-integrin [18]. In addition, integrin-mediated outside-in signaling through Syk, Erk and Akt is deficient in kindlin-3 $-/-$ osteoclasts [17], and targeted knockdown of kindlin-3 leads to reduced alphaMbeta2 (Mac-1)-mediated Syk and Vav signaling, and reduced Rac-1 activation in Mac-1-transfected K562 cells [30]. How kindlin-3 couples to these intracellular signaling pathways remains to be investigated in the future.

Non-integrin related effects of kindlin deficiency

In addition to integrins, kindlin proteins have also been described to interact with binding partners such as integrin linked kinase (ILK) and migfilin and kindlin-3 has been described to interact with receptor for activated C kinase 1 (RACK1) [29, 31]. Interestingly, kindlin-3 deficient mice have been described to have abnormally shaped erythrocytes with disrupted membrane skeletons [32]. As erythrocytes don't have integrins, this finding indicates that kindlin-3 has functions in cells which are not integrin dependent. The molecular targets for such functions of kindlin-3 have so far not been described.

Integrin-independent functions have also been described for other kindlin-family members. Kindlin-2 binds directly to the clathrin heavy chain, which regulates clathrin-dependent vesicle trafficking in endothelial cells in an integrin-independent manner. Therefore, trafficking of CD39 and CD73, which are enzymes involved in ADP/AMP catabolism, is affected in Kindlin2 +/- mice [33]. Kindlin-2 has also been reported to bind to the TGF- β type 1 receptor through its FERM domain and to Smad3 through its N terminus, which mediates this interaction in human kidney tubular epithelial cells [34]. Kindlin-1 and kindlin-2 have also been described to be found in the nucleus of cells; no such localization of kindlin-3 has so far been reported [6].

Conclusions

It is by now well established that kindlin-3 plays a fundamentally important role in integrin regu-

lation in immune cells. Further research is now necessary to reveal the molecular details of this process, as well as defining non-integrin related protein partners for kindlin-3.

Acknowledgements

The work in the authors' laboratories is funded by BBSRC, Arthritis Research UK, Tenovus Scotland, Academy of Finland, Biocentrum Helsinki and the Liv och Hälsa foundation.

Disclosure of conflict of interest

None.

Address correspondence to: Susanna C Fagerholm, Institute of Biotechnology, University of Helsinki, Finland. E-mail: susanna.fagerholm@helsinki.fi

References

- [1] Gahmberg CG, Fagerholm SC, Nurmi SM, Chavakis T, Marchesan S and Gronholm M. Regulation of integrin activity and signalling. *Biochim Biophys Acta* 2009; 1790: 431-444.
- [2] MacPherson M, Lek HS, Morrison V and Fagerholm S. Leukocyte Beta2-Integrins; Genes and Disease. *J Genet Syndr Gene Ther* 2013; 4: 154.
- [3] Nurden AT, Fiore M, Nurden P and Pillois X. Glanzmann thrombasthenia: a review of ITGA2B and ITGB3 defects with emphasis on variants, phenotypic variability, and mouse models. *Blood* 2011; 118: 5996-6005.
- [4] Takala H, Nurminen E, Nurmi SM, Aatonen M, Strandin T, Takatalo M, Kiema T, Gahmberg CG, Ylanne J and Fagerholm SC. Beta2 integrin phosphorylation on Thr758 acts as a molecular switch to regulate 14-3-3 and filamin binding. *Blood* 2008; 112: 1853-1862.
- [5] Moser M, Bauer M, Schmid S, Ruppert R, Schmidt S, Sixt M, Wang HV, Sperandio M and Fassler R. Kindlin-3 is required for beta2-integrin-mediated leukocyte adhesion to endothelial cells. *Nat Med* 2009; 15: 300-305.
- [6] Meves A, Stremmel C, Gottschalk K and Fassler R. The Kindlin protein family: new members to the club of focal adhesion proteins. *Trends Cell Biol* 2009; 19: 504-513.
- [7] Montanez E, Ussar S, Schifferer M, Bosl M, Zent R, Moser M and Fassler R. Kindlin-2 controls bidirectional signaling of integrins. *Genes Dev* 2008; 22: 1325-1330.
- [8] Dowling JJ, Vreede AP, Kim S, Golden J and Feldman EL. Kindlin-2 is required for myocyte elongation and is essential for myogenesis. *BMC Cell Biol* 2008; 9: 36.

Kindlin-3 in immunity

- [9] Dowling JJ, Gibbs E, Russell M, Goldman D, Minarcik J, Golden JA and Feldman EL. Kindlin-2 is an essential component of intercalated discs and is required for vertebrate cardiac structure and function. *Circ Res* 2008; 102: 423-431.
- [10] Pluskota E, Dowling JJ, Gordon N, Golden JA, Szpak D, West XZ, Nestor C, Ma YQ, Bialkowska K, Byzova T and Plow EF. The integrin co-activator kindlin-2 plays a critical role in angiogenesis in mice and zebrafish. *Blood* 2011; 117: 4978-4987.
- [11] Ussar S, Wang HV, Linder S, Fassler R and Moser M. The Kindlins: subcellular localization and expression during murine development. *Exp Cell Res* 2006; 312: 3142-3151.
- [12] Bialkowska K, Ma YQ, Bledzka K, Sossey-Alaoui K, Izem L, Zhang X, Malinin N, Qin J, Byzova T and Plow EF. The integrin co-activator Kindlin-3 is expressed and functional in a non-hematopoietic cell, the endothelial cell. *J Biol Chem* 2010; 285: 18640-18649.
- [13] Yates LA, Fuzery AK, Bonet R, Campbell ID and Gilbert RJ. Biophysical analysis of Kindlin-3 reveals an elongated conformation and maps integrin binding to the membrane-distal beta-subunit NPXY motif. *J Biol Chem* 2012; 287: 37715-37731.
- [14] Svensson L, Howarth K, McDowall A, Patzak I, Evans R, Ussar S, Moser M, Metin A, Fried M, Tomlinson I and Hogg N. Leukocyte adhesion deficiency-III is caused by mutations in KINDLIN3 affecting integrin activation. *Nat Med* 2009; 15: 306-312.
- [15] Malinin NL, Zhang L, Choi J, Ciocea A, Razorenova O, Ma YQ, Podrez EA, Tosi M, Lennon DP, Caplan AI, Shurin SB, Plow EF and Byzova TV. A point mutation in KINDLIN3 ablates activation of three integrin subfamilies in humans. *Nat Med* 2009; 15: 313-318.
- [16] Kuijpers TW, van de Vijver E, Weterman MA, de Boer M, Tool AT, van den Berg TK, Moser M, Jakobs ME, Seeger K, Sanal O, Unal S, Cetin M, Roos D, Verhoeven AJ and Baas F. LAD-1/variant syndrome is caused by mutations in FERMT3. *Blood* 2009; 113: 4740-4746.
- [17] Schmidt S, Nakchbandi I, Ruppert R, Kawelke N, Hess MW, Pfaller K, Jurdic P, Fassler R and Moser M. Kindlin-3-mediated signaling from multiple integrin classes is required for osteoclast-mediated bone resorption. *J Cell Biol* 2011; 192: 883-897.
- [18] Morrison VL, Macpherson M, Savinko T, San Lek H, Prescott A and Fagerholm SC. The beta2 integrin-kindlin-3 interaction is essential for T-cell homing but dispensable for T-cell activation in vivo. *Blood* 2013; 122: 1428-1436.
- [19] Nurmi SM, Autero M, Raunio AK, Gahmberg CG and Fagerholm SC. Phosphorylation of the LFA-1 integrin beta2-chain on Thr-758 leads to adhesion, Rac-1/Cdc42 activation, and stimulation of CD69 expression in human T cells. *J Biol Chem* 2007; 282: 968-975.
- [20] Fagerholm SC, Hilden TJ, Nurmi SM and Gahmberg CG. Specific integrin alpha and beta chain phosphorylations regulate LFA-1 activation through affinity-dependent and -independent mechanisms. *J Cell Biol* 2005; 171: 705-715.
- [21] Bottcher RT, Stremmel C, Meves A, Meyer H, Widmaier M, Tseng HY and Fassler R. Sorting nexin 17 prevents lysosomal degradation of beta1 integrins by binding to the beta1-integrin tail. *Nat Cell Biol* 2012; 14: 584-92.
- [22] Moser M, Nieswandt B, Ussar S, Pozgajova M and Fassler R. Kindlin-3 is essential for integrin activation and platelet aggregation. *Nat Med* 2008; 14: 325-330.
- [23] Willenbrock F, Zicha D, Hoppe A and Hogg N. Novel automated tracking analysis of particles subjected to shear flow: kindlin-3 role in B cells. *Biophys J* 2013; 105: 1110-1122.
- [24] Moretti FA, Moser M, Lyck R, Abadier M, Ruppert R, Engelhardt B and Fassler R. Kindlin-3 regulates integrin activation and adhesion reinforcement of effector T cells. *Proc Natl Acad Sci U S A* 2013; 110: 17005-10.
- [25] Cohen SJ, Gurevich I, Feigelson SW, Petrovich E, Moser M, Shakhar G, Fassler R and Alon R. The integrin co-activator Kindlin-3 is not required for lymphocyte diapedesis. *Blood* 2013; 122: 2609-17.
- [26] Gruda R, Brown AC, Grabovsky V, Mizrahi S, Gur C, Feigelson SW, Achdout H, Bar-On Y, Alon R, Aker M, Davis DM and Mandelboim O. Loss of kindlin-3 alters the threshold for NK cell activation in human leukocyte adhesion deficiency-III. *Blood* 2012; 120: 3915-3924.
- [27] Lefort CT, Rossaint J, Moser M, Petrich BG, Zarbock A, Monkley SJ, Critchley DR, Ginsberg MH, Fassler R and Ley K. Distinct roles for talin-1 and kindlin-3 in LFA-1 extension and affinity regulation. *Blood* 2012; 119: 4275-4282.
- [28] Wernimont SA, Wiemer AJ, Bennin DA, Monkley SJ, Ludwig T, Critchley DR and Huttenlocher A. Contact-dependent T cell activation and T cell stopping require talin1. *J Immunol* 2011; 187: 6256-6267.
- [29] Feng C, Li YF, Yau YH, Lee HS, Tang XY, Xue ZH, Zhou YC, Lim WM, Cornvik TC, Ruedl C, Shochat SG and Tan SM. Kindlin-3 mediates integrin alphaLbeta2 outside-in signaling, and it interacts with scaffold protein receptor for activated-C kinase 1 (RACK1). *J Biol Chem* 2012; 287: 10714-10726.
- [30] Xue ZH, Feng C, Liu WL and Tan SM. A role of kindlin-3 in integrin alphaMbeta2 outside-in signaling and the Syk-Vav1-Rac1/Cdc42 signaling axis. *PLoS One* 2013; 8: e56911.

Kindlin-3 in immunity

- [31] Tu Y, Wu S, Shi X, Chen K and Wu C. Migfilin and Mig-2 link focal adhesions to filamin and the actin cytoskeleton and function in cell shape modulation. *Cell* 2003; 113: 37-47.
- [32] Kruger M, Moser M, Ussar S, Thievensen I, Luber CA, Forner F, Schmidt S, Zanivan S, Fassler R and Mann M. SILAC mouse for quantitative proteomics uncovers kindlin-3 as an essential factor for red blood cell function. *Cell* 2008; 134: 353-364.
- [33] Pluskota E, Ma Y, Bledzka KM, Bialkowska K, Soloviev DA, Szpak D, Podrez EA, Fox PL, Hazen SL, Dowling JJ, Ma YQ and Plow EF. Kindlin-2 regulates hemostasis by controlling endothelial cell-surface expression of ADP/AMP catabolic enzymes via a clathrin-dependent mechanism. *Blood* 2013; 122: 2491-2499.
- [34] Wei X, Xia Y, Li F, Tang Y, Nie J, Liu Y, Zhou Z, Zhang H and Hou FF. Kindlin-2 Mediates Activation of TGF-beta/Smad Signaling and Renal Fibrosis. *J Am Soc Nephrol* 2013; 24: 1387-1398.
- [35] Monkley SJ, Zhou XH, Kinston SJ, Giblett SM, Hemmings L, Priddle H, Brown JE, Pritchard CA, Critchley DR and Fassler R. Disruption of the talin gene arrests mouse development at the gastrulation stage. *Dev Dyn* 2000; 219: 560-574.