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Quick guide

Ajuba proteins

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What are Ajuba proteins? The Ajuba adaptor proteins are characterized by the presence of three highly related tandem LIM domains at their carboxyl terminus (the LIM region), and a variable proline-rich amino-terminal preLIM region (Figure 1). Consistent with their ability to shuttle between the nucleus and cytoplasm, Ajuba proteins contain a nuclear export sequence (NES) in their preLIM region, while the LIM region directs nuclear localization. The mammalian Ajuba family includes three members with overlapping tissue/cell expression: Ajuba, LIM domain containing protein 1 (LIMD1), and Wilms tumor 1 interacting protein (WTIP). *Drosophila* only has a single Ajuba family member, *dJub*, which is most closely related in organization to mammalian LIMD1.

What are LIM domains? LIM domains are cysteine-rich, double zinc-finger domains originally identified 25 years ago in the nuclear homeobox transcription factors LIN-11, Isl1 and MEC3, which regulate cell fate during development. These domains are now recognized as protein–protein interaction domains that are as abundant as common protein-interaction motifs, such as SH2 and SH3 domains. The modular nature of LIM domains and the fact that many proteins contain multiple LIM domains allow for interactions between a diverse array of proteins in different subcellular locations (cell surface, actin cytoskeleton, cytosol, and nucleus). LIM domains can also bind to LIM domains within other proteins. There is no consensus LIM domain recognition sequence in proteins, implying that these domains may recognize some common structural feature of proteins.

What are the cellular and biological functions of the Ajuba proteins? Ajuba proteins participate in a diverse array of cellular processes — cell–cell adhesion, cell–matrix adhesion, cell migration, cell proliferation and survival, and mitotic cytokinesis. Due to functional redundancy and overlapping expression, the organismal functions of the Ajuba protein family in mammals has not been determined but in *Drosophila* the single Ajuba gene *dJub* is essential for embryonic development. Selective tissue deletion of *dJub* revealed that dJub is critical for the regulation of organ size as a negative regulator of the Hippo signaling pathway, while in neuroblasts dJub maintains centrosome structure and function. In zebrafish *AJUBA* is critical for proper heart development by coupling retinoic acid signaling with the transcriptional activity of another nuclear LIM protein, Isl1. In human pathology, Ajuba proteins have been implicated in tumor development as tumor suppressors. LIMD1 can function as a co-repressor of the Retinoblastoma protein (pRB). The *LIMD1* gene is located at chromosome 3p21.3, a region commonly deleted in lung cancer, and *LIMD1* null mice cooperate with oncogenic Ras in lung cancer initiation. Indeed, the *LIMD1* gene is frequently inactivated in human lung cancers.

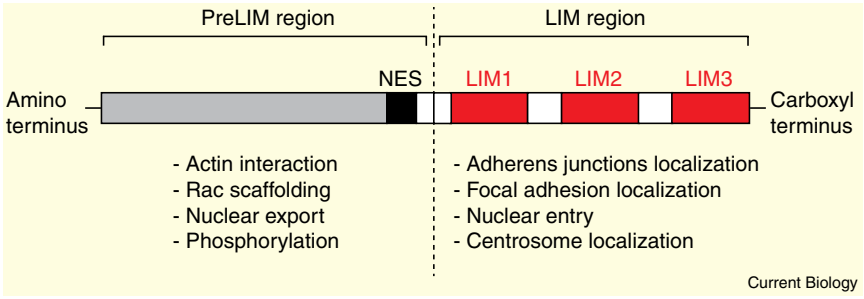

Figure 1. Ajuba protein domains.
Schematic figure depicting how different regions of Ajuba proteins contribute to differing subcellular localizations.

What is the molecular basis of Ajuba protein function? By virtue of their structure and subcellular localization, Ajuba proteins function as adapters and scaffolds in the transduction of external signals such as growth factor, cell–cell and cell–matrix adhesion, and mechanical signals. They can couple different signaling pathways through distinct interactions with the preLIM and LIM regions. As proteins that shuttle between different subcellular compartments, Ajuba proteins are ideally suited to relay signals between the cell surface and nucleus. Ajuba proteins may exist in a closed preLIM–LIM interacting inactive conformation at basal states that is opened ('activated') following phosphorylation of the preLIM region in response to signals that allow for interaction with various binding partners. Phosphorylation also stabilizes the protein.

What are the nuclear functions of Ajuba proteins? In the nucleus Ajuba proteins function as transcriptional co-repressors. For example, they assemble a Snail1 repressor complex, by coupling Snail1 (via the LIM region) with the chromatin-remodeling enzyme PRMT5 (preLIM region), that induces an epithelial–mesenchymal transition that controls neural crest development in *Xenopus*. In developing zebrafish hearts Ajuba links retinoic acid signaling to Isl1 to repress Isl1 transcriptional activity and restrict progenitor cell specification and proliferation. Retinoic acid signals also increase the expression of Ajuba and its nuclear entry.

What are the cytoplasmic functions of Ajuba proteins? Ajuba proteins localize to centrosomes. There they interact with the Hippo pathway components LATS1/2 and also with Aurora A kinases, which are important for centrosome integrity and function. In *Drosophila*, loss of dJub in neural stem cells results in centrosome defects and aberrant mitotic spindles.

What are the cell-surface functions of Ajuba proteins? In epithelial cells, Ajuba proteins are recruited to newly forming adherens junctions through an interaction with α-catenin (via the LIM region) and as a result stabilize junctions by contributing to the coupling of cadherin-based adhesive complexes to the actin cytoskeleton (via the preLIM region). Ajuba acts as a scaffold that concentrates active Rac (via the preLIM

region) at sites of cell–cell contact: Rac does not interact with LIMD1 even though LIMD1 is recruited to adherens junctions.

What is the link between Ajuba proteins and the Hippo signaling pathway? Through their interaction with LATS/Wts kinases (via the LIM region), Ajuba proteins and dJub are negative regulators of the Hippo signaling pathway. LATS/Wts kinases are components of the conserved Hippo core kinase complex that directly phosphorylate the transcriptional activator YAP/Yki, leading to its nuclear exclusion and degradation. Precisely where in the cell, how, and during what biological circumstances Ajuba proteins inhibit LATS/Wts activity is not fully appreciated, but during *Drosophila* wing development dJub regulates cytoskeletal-tension-induced proliferation by inhibiting the Hippo pathway.

Where can I find out more?

Das Thakur, M., Feng, Y., Jagannathan, R., Seppa, M.J., Skeath, J.B., and Longmore, G.D. (2010). Ajuba LIM proteins are negative regulators of the Hippo signaling pathway. Curr. Biol. *7*, 657–662.

Hirota, T., Kunitoku, N., Sasayama, T., Marumoto, T., Zhang, D., Nitta, M., Hatakeyama, K., and Saya, H. (2003). Aurora-A and an interacting activator, the LIM protein Ajuba, are required for mitotic commitment in human cells. Cell *5*, 585–598.

Kadrmas, J., and Beckerle, M.C. (2004). The LIM domain: from the cytoskeleton to the nucleus. Nat. Rev. Mol. Cell Biol. *5*, 920–931.

Kanungo, J., Pratt, S., Marie, H., and Longmore, G.D. (2000). Ajuba, a cytosolic LIM protein, shuttles into the nucleus and affects embryonal cell proliferation and fate decisions. Mol. Biol. Cell *10*, 3299–3313.

Langer, E.M., Feng, Y., Zhaoyuan, H., Rauscher, F.J., 3rd, Kroll, K.L., and Longmore G.D. (2008). Ajuba LIM proteins are snail/slug corepressors required for neural crest development in Xenopus. Dev. Cell *3*, 424–436.

Nola, S., Daigaku, R., Smolarczyk, K., Carstens, M., Martin-Martin, B., Longmore, G., Bailly, M., and Braga, V.M.M. (2011). Ajuba is required for Rac activation and maintenance of E-cadherin adhesion. J. Cell Biol. *5*, 855–871.

Rauskolb, C., Sun, S., Sun, G., Pan, Y., and Irvine, K.D. (2014). Cytoskeletal tension inhibits Hippo signaling through an Ajuba-Warts complex. Cell *158*, 143–156.

Sharp, T.V., Al-Attar, A., Foxler, D., Ding, L., de, A., Vallim, T., Zhang, Y., Nijmeh, H., Webb, T., Nicholson, A., *et al*. (2008). The chromosome 3p21.3-encoded gene, LIMD1, is a critical tumor suppressor involved in human lung cancer development. Proc. Natl. Acad. Sci. USA *50*, 19932–19937.

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Facultative parthenogenesis in a critically endangered wild vertebrate

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Facultative parthenogenesis — the ability of sexually reproducing species to sometimes produce offspring asexually — is known from a wide range of ordinarily sexually reproducing vertebrates in captivity, including some birds, reptiles and sharks [1–3]. Despite this, free-living parthenogens have never been observed in any of these taxa in the wild, although two free-living snakes were recently discovered each gestating a single parthenogen — one copperhead (*Agkistrodon contortrix*) and one cottonmouth (*Agkistrodon piscivorus*) [1]. Vertebrate parthenogens are characterized as being of the homogametic sex (e.g., females in sharks, males in birds) and by having elevated homozygosity compared to their mother [1–3], which may reduce their viability [4]. Although it is unknown if either of the parthenogenetic snakes would have been carried to term or survived in the wild, facultative parthenogenesis might have adaptive significance [1]. If this is true, it is reasonable to hypothesize that parthenogenesis would be found most often at low population density, when females risk reproductive failure because finding mates is difficult [5]. Here, we document the first examples of viable parthenogens living in a normally sexually reproducing wild vertebrate, the smalltooth sawfish (*Pristis pectinata*). We also provide a simple approach to screen any microsatellite DNA database for parthenogens, which will enable hypothesis-driven research on the significance of vertebrate parthenogenesis in the wild.

The smalltooth sawfish is a large, critically endangered ray that is estimated to have declined to 1–5% of its population size in 1900. It currently occurs primarily in southwest Florida, U.S. [6], where it may have stabilized and started to recover (http://www.iucnredlist.org/details/18175/0). Between 2004 and 2013, 190 individuals ranging in stretched total length (STL) from 67.1 to 381 cm were sampled, tagged, and released in the Caloosahatchee River, Peace River and Ten Thousand Islands regions. Sixteen microsatellite loci were used to genotype these individuals (Supplemental Information). These loci had from 4 to 40 alleles (mean = 20) and conformed to Hardy-Weinberg equilibrium expectations after correction for multiple tests (Supplemental Information). The program STORM [7] was used to calculate the internal relatedness (IR) of each individual, a parameter that expresses the relatedness of the individual's parents [8]. Outbred individuals will have an IR close to zero while individuals derived from close kin mating are expected to exhibit IR values from ~0.25 to 0.50 (i.e., indicating that the individual's parents were half or full-siblings, respectively). An IR value close to or equal to 1 indicates near or complete homozygosity across surveyed loci, which is consistent with all known cases of facultative parthenogenesis in vertebrates [1–3].

The mean IR of sampled individuals was 0.033 (s.d. = 0.199, with >85% of individuals having an IR <0.10; Figure 1), indicating that mating pairs were typically unrelated. Seven outliers (3.7% of the sample) with IR values ranging from 0.84 to 1.0 were found, with two individuals being homozygous at all loci and five being homozygous at all loci but one or two (Figure 1). All of these individuals were female, even though the overall sample sex ratio excluding these individuals was close to unity (1 male to 1.12 females). Five of the outlier individuals were captured in the Peace River in 2011 (n = 4) and 2012 (n = 1). Their size at capture indicated they were all born in 2011 [9] and their genotypes indicate they were full siblings (Supplemental Information). We consider these five individuals to be members of a single brood of parthenogenetic offspring, which has previously been observed in captive white-spotted bamboo sharks, *Chiloscyllium plagiosum* (brood

