CELL BIOLOGY

A ligand for ALK

Greg Lemke* and Erin D. Lew

The receptor tyrosine kinase ALK (anaplastic lymphoma kinase), as its name suggests, is aberrantly activated, mutated, or abundant in multiple cancers. Although widely studied in the context of cancer, the ligand that normally binds to and activates ALK in vertebrates has remained elusive. In this issue of *Science Signaling*, Murray and colleagues identify heparin as an ALK ligand.

During the heyday of the molecular cloning of genes encoding cell surface receptors, there were many "orphans." These were newly identified proteins for which an activating agonist, or ligand, had yet to be identified. For receptor tyrosine kinases (RTKs), which are cell surface receptors that carry a protein tyrosine kinase within their cytoplasmic domains, this era has largely closed. We now know, for example, that fibrillar collagens are ligands for the discoidin domain receptor (DDR) family (*1*), that the ephrins are ligands for the large family of Eph receptors (*2*), and that protein S (Pros1) and growth arrest–specific 6 (Gas6) are ligands for the Tyro3, Axl, and Mer (TAM) family receptors (*3*). However, there are a few remaining RTK orphans, and among them is anaplastic lymphoma kinase (ALK) (*4*). In this issue of *Science Signaling*, Murray and colleagues provide compelling evidence that heparin is capable of binding to and activating vertebrate ALK (*5*).

ALK has been extensively studied in the context of cancer, particularly non–small-cell lung carcinoma and anaplastic large-cell lymphoma, with which it was first associated. These and other cancers may be driven by gene fusion events between the tyrosine kinase domain of ALK and various protein-coding domains, by mutations in full-length ALK, or by increased abundance of the ALK protein. There are currently nearly 2000 citations in PubMed identified by a query for "anaplastic lymphoma kinase." However, despite this intense interest, the identity of the true ligand(s) for vertebrate ALK has been difficult to pin down. Although the secreted protein Jelly belly was found to act as a ligand for *Drosophila* ALK (*6*), efforts to assign the heparin-binding proteins Pleiotrophin and Midkine as ligands for vertebrate ALK (*7*, *8*) appear to have been false starts. In this issue of *Science Signaling*, Murray and colleagues (*5*) focused not on a heparin-binding protein but on heparin itself.

Heparin is a family of highly acidic, sulfated glycosaminoglycan polymers of disaccharide subunits (usually of 2-O-sulfated iduronic acid and 6-O-sulfated, N-sulfated glucosamine) of varying chain lengths. It is prominent in the secretory granules of mast cells and basophils, but its very closely related sulfated disaccharide cousin, heparan sulfate, is widely distributed throughout body tissues in the form of heparan sulfate proteoglycans (HSPGs). Prominent HSPGs include the syndecans and glypicans. Murray *et al*. (*5*) found that heparin binds, presumably in large part through electrostatic interactions, specifically to a highly basic region at the N terminus of the ALK extracellular domain (ectodomain). This region is conserved among the ectodomains of all vertebrate ALK orthologs but is missing from the *Drosophila* protein. They found that this basic region was required both for heparin binding and for heparin activation of ALK in NB-1 neuroblastoma cells, which have endogenous ALK. The addition of heparin alone to the media of cultured NB-1 cells was sufficient to strongly activate the tyrosine kinase activity of ALK, presumably by facilitating dimerization or higher-order oligomerization of the receptor.

Murray *et al*. (*5*) also found that the affinity of heparin binding, the ability of heparin to activate ALK, and the stoichiometry of the heparin-ALK interaction were all a function of heparin chain length (Fig. 1). Heparin polymers composed of eight or fewer disaccharide units were relatively weak ALK activators in their NB-1 assays and displayed monovalent ALK-heparin binding, whereas polymers of 15 or more disaccharide subunits were strong ALK activators and displayed divalent (or for really long heparin chains, tetravalent) ALK-heparin binding.

Heparin, heparan sulfate, and HSPGs are not unknown players to RTK aficionados. These molecules serve as critical co-ligands for signaling by fibroblast growth factors (FGFs) through FGF receptors (*9*). Heparin, heparan sulfate, or, more commonly, the heparan sulfate chains of HSPGs bind both to FGFs and to FGFRs through a positively charged crevice formed by the interaction of two FGFR ectodomains with two FGF molecules, playing a key role in the dimerization of the receptors (*10*, *11*). The effective FGF signaling complex is therefore a 2:2:2 ternary structure composed of two FGF ligands, an FGFR dimer, and the sulfated disaccharide chains. The presence of heparin or heparan sulfate–like molecules in this ternary

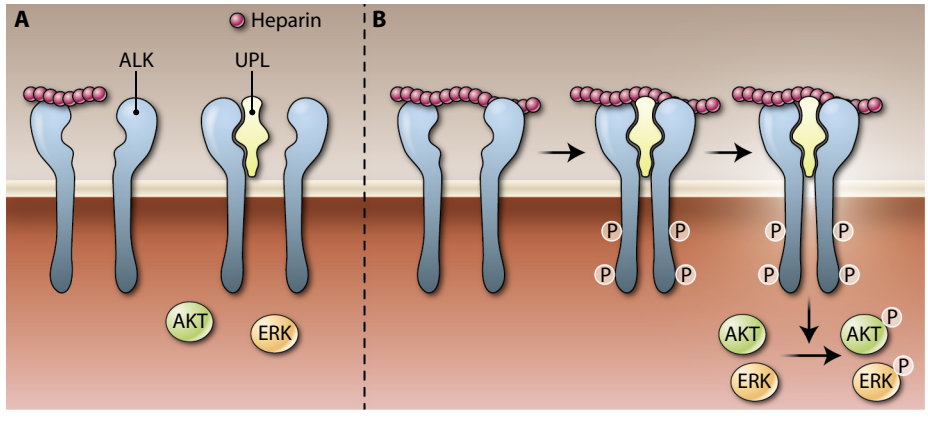

Fig. 1. Heparin activation of ALK. (**A**) Heparin chain lengths of eight disaccharide units bind to the N terminus of ALK but are weak drivers of receptor multimerization and weak receptor activators. An unidentified protein ligand (UPL) may bind independently to the ALK ectodomain. (**B**) Heparin chain lengths of 15 or more disaccharide units bind tightly to ALK and strongly induce receptor multimerization and activation. The putative UPL could bind both ALK and heparin in a manner analogous to that seen for FGF binding to both the FGF receptor and heparin.

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complex typically potentiates the strength of FGF signaling through FGFRs by at least an order of magnitude.

These features of FGF and FGFR signaling raise the possibility that heparin and closely related molecules, although clearly binding to and stimulating the activity of ALK in the systems used by Murray and colleagues, may not be the only ligands for ALK. The authors noted that ALK shares a glycine-rich region and an EGF-like repeat with the ectodomain of another long-orphaned and largely neglected RTK: leukocyte tyrosine kinase (LTK) (*12*). This related RTK lacks the basic N-terminal region through which ALK binds to heparin (*5*). Therefore, it is possible that the ectodomain regions conserved in LTK and ALK are involved in the binding of a common protein ligand or closely related ligands, which, in concert with an HSPG, activate these two receptors (Fig. 1). If this is the case, then NB-1 cells presumably must make this protein. Recently, an extracellular proteome screening approach was used to identify a reasonable candidate for just such a putative ligand for LTK (*13*).

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