**AKAP: Coming to a Location Within You**

**Location Matters: Localization of Protein Kinase A and DPY-30**

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**Abstract**

Signal transduction is an essential process in cells. One critical signaling molecule, protein kinase A (PKA), phosphorylates target proteins, thereby changing their conformations and modifying their functions. PKA is a component of multiple signaling pathways that regulate a variety of proteins. Since the broad substrate specificity of PKA can lead to phosphorylation of unintended proteins, PKA activity must be limited to specific times and places. Protein kinase anchoring proteins (AKAPs) bind and help localize PKA to specific areas. The RIIa domain in PKA provides a shallow groove for an amphipathic helix of AKAP to bind via interactions of hydrophobic side chains. A similar binding motif is found in the DPY-30 domain, which suggests this domain may also play a localization role. The ability of AKAP to interact with PKA and regulate its activity is critical for many cellular responses. The ability of a cell to localize proteins containing a DPY-30 domain may also be important for proper function. If localization is disrupted, serious problems like heart disease and cancer may result. To further understand the impact of structural interactions on localization, physical models of RIIa, DPY-30, and AKAP amphipathic helix have been designed and built by the Cedarburg High School SMART (Students Modeling a Research Topic) Team using 3D printing technology.

**If molecules are not in their proper location within a cell at the right time, disease may result.**

- Protein kinase A (PKA) is an enzyme involved in a multitude of signaling pathways in cells, including those that regulate heartbeat. PKA catalyzes the addition of a phosphate group to another protein, thereby changing its shape and regulating its function. If PKA is not localized properly within a cell, then PKA activity and its signaling are not as precise.
- For example, heart failure is linked to hyperphosphorylation by PKA of a receptor involved in Ca^{2+} transport into cardiac muscle. In addition, a correlation is shown to exist between the amount of phosphorylation of the receptor and the degree to which the heart is affected (Reiken et al., 2003).
- DPY-30 domain is found in many proteins, including DPY-30 in Set-1 histone methyltransferase (MT) complex. One way of regulating gene expression is to methylate the histones that package DNA. Depending on which chromatin regions where associated histones are methylated, transcription rates may increase or decrease.
- Methylation of histones is but one of several possible histone modifications, and it has been suggested that this variety may arise from the replacement of histone H3 with a variant (replacement) histone H3.3. Histone replacement occurs in transcriptionally active regions of chromatin and increased levels of histone replacement is observed in leukemia.
- By disrupting the localization of DPY-30 it may be possible to prevent the assembly or nuclear transport of the MT system, thus suppressing the histone replacement that occurs in leukemia.

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**Figure 1A.** Because PKA is involved in so many different signaling pathways, it is critical that it be active at the right place and right time within a cell.

**Figure 1B.** The MT complex consists of a variety of polypeptides, including mixed-lineage leukemia (MLL) proteins and DPY30 that contains a DPY-30 domain. This molecule complex methylates histone 3 and acetylates histone 4. The modifications loosen the packing of the chromatin within a nucleosome, activating transcription. During transcriptional elongation, the MT complex associates and “rides” with RNA polymerase II, thereby spreading the histone modification pattern along an actively transcribed region. Note that DPY-30 and a few subunits are not illustrated and that this diagram is not to scale.

**Figure 2.** The specific localization of PKA is facilitated by the interaction of hydrophobic residues of a shallow binding groove in the RIIa dimer (blue) with the hydrophobic residues of an amphipathic helix (brown). (Kinderman, 2006)

**Figure 3.** RIIa of PKA (views A and E) and DPY-30 domains (views B and F) share structural similarities (Kinderman et al., 2006; Wang et al., 2009). The RIIa dimer is complexed with the amphipathic helix (AH) from an AKAP (C), and a possible interaction between AH and DPY-30 is shown (D). The AH from the RIIa-AH complex is isolated in view G. The structural feature of the DPY-30-domain-binding peptide remains unknown. In each representation, the space-filled residues colored yellow or orange are hydrophobic amino acids involved in the molecular interactions.

**Figure 4.** DPY-30 domain cross-reacts with the amphipathic helix (AH) of a flagellar AKAP (A kinase anchoring protein) known to bind the RIIa domain of PKA. The lane labeled Pre is an extract of bacteria engineered to express either RIIa and AH (left) or DPY-30 and AH (right). The lane labeled Post contains the flow through after the bacterial extract was subjected to Ni-NTA affinity purification. The co-expressed AH and RIIa were depleted in the flow through and co-purified in the elute. Similarly, co-expressed DPY-30 domain and the AH were purified together as well. Thus the same AH can recognize both the RIIa and DPY-30 domain.

**Conclusion**

AH from AKAP binds RIIa-like domains from various proteins. Based on the structural similarity (Figure 3) and cross recognition (Figure 4), we predict that the binding sites for DPY-30 and RIIa domains will share sequence or structural similarities.

By studying the interaction of the DPY-30 domain with AH, it may one day be possible to develop a therapeutic agent that could be used to treat leukemia by disrupting interactions important in localizing MT.

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**References**


