A molecular thermometer

Temperature dependent conformational dynamics of SR-Protein Kinases

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In Short

- Protein kinase
- Temperature sensitivity
- Conformational dynamics

We recently reported that body temperature cycles control a rhythmic alternative splicing program in mammals [1,2]. Body temperature cycles drive rhythmic SR protein phosphorylation resulting in a concerted splicing switch in a large group of functionally related genes. However, the temperature sensing mechanism upstream of SR protein phosphorylation remains elusive. In the present study we are investigating the molecular basis for the cellular thermometer that is sensitive enough to use subtle changes in body temperature as input to produce altered alternative splicing as output.

The phosphorylation state of SR proteins is controlled by an interplay of kinases and phosphatases SR proteins are hyperphosphorylated in colder temperatures and vice versa in heat. SR protein phosphorylation is controlled by two classes of kinases, SRPKs (SR protein kinases, SRPK1-3) and CLKs [3,4]. Overexpression and knockdown experiments suggested a prominent role of Cdc2-like kinases (CLKs), known SR protein specific kinases, in regulating temperature dependent SR protein phosphorylation

Using recombinant CLK proteins in in vitro kinase assays to investigate the role of these kinases in more detail revealed temperature-dependent activity. Consistent with higher SR-protein phosphorylation at lower temperatures, CLK autophosphorylation as well as phosphorylation of an RS-repeat was more efficient at lower temperatures. Remarkably, we observe a linear increase of auto- and substrate phosphorylation within a temperature range of 2 ℃ between 38 ℃ and 36 ℃, reflecting the physiological circadian body temperature range in mammals. The sensitivity of CLKs to changes in the physiologically relevant temperature range and its surprising increase in activity at lower temperatures provide a potential mechanism for time of the day dependent splicing regulation in mammals. Our current work is focused on investigating the structural basis and the evolutionary conservation of CLK temperature sensitivity.

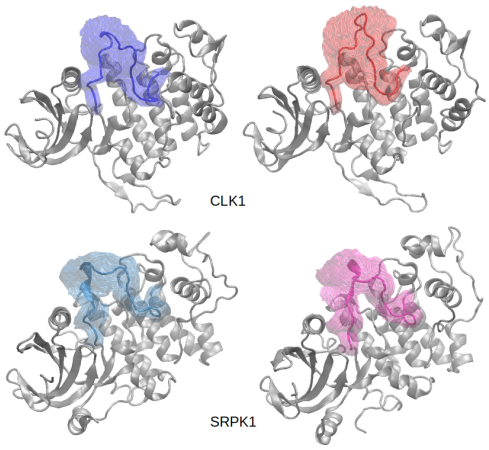

Figure 1: *Flexibility of the protein kinases as obtained from simulations at low (20 ℃, blue) and high (40 ℃, red) temperatures.*

Our molecular dynamics simulations thus far show a different temperature-dependence in the conformational behaviour of the two protein kinases CLK1 and SRPK1. In particular one region, termed the activation loop, explores a significantly larger space at high temepratures in CLK1. For SRPK1, in contrast, the conformational flexibility of this region is comparable at the two temperature (see Figure 1).

The aim of this project is to further test the hypothesis of the activation loop as the thermo-sensor and thus responsible for temperature-dependent kinase activity. We will furthermore elucidate the origin of the different temperature-dependent conformational felxibility of the activation loops in the two protein kinases CLK and SRPK.

More Information

[1] M. Preussner et al., Body Temperature Cycles Control Rhythmic Alternative Splicing in Mammals. *Molecular cell* 67, 433-446.e434 (2017)

[2] M. Preussner, F. Heyd, Temperature-controlled Rhythmic Gene Expression in

Endothermic Mammals: All Diurnal Rhythms are Equal, but Some are Circadian. *BioEssays : news and reviews in molecular, cellular and developmental biology* 40, e1700216 (2018)

[3] G. Gourisankar, A. J. A., Phosphorylation mechanism and structure of serine-arginine protein kinases. *The FEBS Journal* 278, 587-597 (2011).

[4] D. Dominguez et al., An extensive program of periodic alternative splicing linked to cell cycle progression. *eLife* 5, (2016).