P-TEFb activation during cardiomyogenesis

G. Contini, E. Mura, I. Marchesi, L. Bagella

Department of Biomedical Sciences, Division of Biochemistry and Biophysics, National Institute of Biostructures and Biosystems, University of Sassari, Italy

The elongation stage of eukaryotic mRNA transcription represents an important regulatory step in the control of gene expression. Positive transcription elongation factor b (P-TEFb) is required for RNA polymerase II (Pol II) to make the transition from abortive to productive elongation via the phosphorilation of its carboxyl-terminal domain (CTD) [1]. In mammals, the CTD comprises 52 repeats of an evolutionally conserved serine-rich heptapeptide, Tyr-Ser-Pro-Thr-Ser-Pro-Ser. P-TEFb induces phosphorylation at Ser2 [2].

P-TEFb is comprised of Cyclin dependent kinase 9 (Cdk9), a member of the cyclin dependent kinase family, and one of four possible cyclin partners cyclin T1, T2a, T2b or K [3]. Cdk9/cyclinT complexes are not a general basal factor, but depending on cell types, they could represent promoter-specific transcriptional elongation complexes. In fact, the role of cdk9 in modulating promoter-restricted transcription appears to be dependent on the sequence-specific factor recruiting the kinase, and on cdk9's ability to associate with different regulatory subunits, such as cyclin T1 and cyclin T2 [4]. Recently, we found that Cdk9 plays a crucial role during myogenesis.

Heart failure results from a variety of cardiovascular disorders including myocardial infarction and hypertension, and it is a principal cause of death and disability in humans [5]. A major morphogenic change in failing hearts is the hypertrophy of each cardiomyocyte, an increase in its cell's volume [6]. Hypertrophic growth is characterized by a global increase in RNA and protein content per cell and by changes in specific gene expressions controlled by a subset of hypertrophy-responsive transcription factors [7, 8]. Moreover, recent studies have shown that hypertrophic signals cause an increment of phosphorylation of RNA polymerase II, mediated by P-TEFb [9].

These studies and earlier works, showing a role of cdk9 in skeletal muscle differentiation, suggest a possible involvement of this kinase during cardiomyogenesis.

In our study GTR1 embryonic stem cells was induced to differentiate into cardiomyocyte. Preliminary data suggest a functional activation of cdk9, in fact, immunoblot analysis shows an increment of fosforilation of RNA polymerase II at level of serine 2. However Cdk9 and cyclin T1 transcript and protein levels, analyzed by Real-Time PCR and immunoblotting at several stage of differentiation, did not show any variations.

Further studies are actually in progress to better understand the role of cdk9 and its regulatory subunits during cardiomyogenesis, and its recruitment by cardio-specific transcription factors.

References

[1] Shim E.Y., Walker A.K., Shi Y., Blackwell T. K. 2002. CDK-9/cyclin T (P-TEFb) is required in two postinitiation pathways for transcription in the C. elegans embryo. Genes Dev. 16: 2135-2146.

[2] Dahmus M.E. 1996. Reversible phosphorylation of the C-terminal domain of RNA polymerase II. J. Biol. Chem. 271: 19009–19012.

[3] Price D.H. 2000. P-TEFb, a cyclin-dependent kinase controlling elongation by RNA polymerase II. Mol. Cell Biol. 20: 2629-2634.

[4] Simone C., Giordano A. 2001. Activation and function of cyclin T–Cdk9 (positive transcription elongation factor-b) in cardiac muscle-cell hypertrophy. Front. Biosci. 6: D1073–D1082.

[5] Dominguez L.J., Parrinello G., Amato P., Licata G. 1999. Trends of congestive heart failure epidemiology: contrast with clinical trial results. Cardiologia 44: 801-808.

[6] Verdecchia P., Carini G., Circo A., Dovellini E., Giovannini E., Lombardo M., Solinas P., Gorini M., Maggioni A.P. and MAVI (MAssa Ventricolare sinistra nell'Ipertensione) Study Group. 2001. Left ventricular mass and cardiovascular morbidity in essential hypertension: the MAVI study. J. Am. Coll. Cardiol. 38(7): 1829-1835.

[7] Akazawa H., Komuro I. 2003. Roles of Cardiac Transcription Factors in Cardiac Hypertrophy. Circulation Research. 92: 1079-1088.

[8] Temsah R., Nemer M. 2005. GATA factors and transcriptional regulation of cardiac natriuretic peptide genes Regul. Pept. 128: 177-185.

[9] Sano M., Abdellatif M., Oh H., Xie M., Bagella L., Giordano A., Michael L.H., Demayo F.J., Schneider M.D. 2002. Activation and function of cyclin T–Cdk9 (positive transcription elongation factor-b) in cardiac muscle-cell hypertrophy. Nat. Med. 8: 1310-1317.