

**A STUDY OF THE PHOSPHORYLATION OF *DROSOPHILA*
MELANOGASTER NUCLEAR LAMINS DURING BOTH INTERPHASE
AND MITOSIS**

RYSZARD RZEPECKI^{1,2} and PAUL A. FISHER¹

¹The Department of Pharmacological Sciences, University Medical Center, The State University of New York at Stony Brook, Stony Brook, NY 11794-8651, USA, ²Institute of Biochemistry and Molecular Biology, University of Wrocław, Przybyszewskiego 63/77, 51-148 Wrocław, Poland

In order to study the phosphorylation of *D. melanogaster* nuclear lamins *in vivo*, we used K_c tissue culture cells. K_c cells contain products of both lamin genes – the lamin Dm₀ gene encoding constitutive polypeptides expressed in almost all cell types and the developmentally-regulated lamin C gene. We grew K_c cells in a low phosphate medium and labelled them with [³²P]H₃PO₄. To obtain mitotic cells, we used vinblastine to arrest the cells in metaphase. Cells were collected, washed, lysed and the resultant extracts fractionated in the presence of protein phosphatase inhibitors. *D. melanogaster* proteins were then denatured by boiling in SDS plus DTT, followed by immunoaffinity chromatography and SDS-PAGE purification. As anticipated, we found that a CNBr fragment derived from the N-terminal part of lamin Dm₀-derivatives (amino acid residues 2-158; fragment A) was phosphorylated during both interphase and mitosis. Interphase but not mitotic phosphorylation was found on an internal CNBr fragment (derived from the end of the central rod domain and the first part of the C-terminal lamin tail; amino acid residues 385-548; fragment D). Interphase-only phosphorylation was also detected on another CNBr fragment derived from the extreme C-terminal portion of lamin Dm₀-derivatives (amino acid residues 549-620; fragment E). To supplement these data, we used 2-D tryptic peptide mapping followed by phosphorImager analysis. We routinely detected at least seven ‘spots’ derived from interphase lamins but only a single mitotic lamin phosphopeptide.