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Research Article

Cyclic AMP regulates the expression and nuclear translocation of RFC40 in MCF7 cells

Rakhee S. Gupte[a,*], *Valerie Sampson*[a], *Frank Traganos*[b], *Zbigniew Darzynkiewicz*[b], *Marietta Y.W.T. Lee*[a]

[a]*Department of Biochemistry and Molecular Biology, New York Medical College, Valhalla, NY 10595, USA*
[b]*Brander Cancer Research Institute, New York Medical College, Valhalla, NY 10595, USA*

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ABSTRACT

We have previously shown that the regulatory subunit of PKA, RIα, functions as a nuclear transport protein for the second subunit of the replication factor C complex, RFC40, and that this transport appears to be crucial for cell cycle progression from G1 to S phase. In this study, we found that N^6-monobutyryl cAMP significantly up-regulates the expression of RFC40 mRNA by 1.8-fold and its endogenous protein by 2.3-fold with a subsequent increase in the RIα-RFC40 complex formation by 3.2-fold. Additionally, the nuclear to cytoplasmic ratio of RFC40 increased by 26% followed by a parallel increase in the percentage of S phase cells by 33%. However, there was reduction in the percentage of G1 cells by 16% and G2/M cells by 43% with a concurrent accumulation of cells in S phase. Interestingly, the higher percentage of S phase cells did not correlate with a parallel increase in DNA replication. Moreover, although cAMP did not affect the expression of the other RFC subunits, there was a significant decrease in the RFC40-37 complex formation by 81.3%, substantiating the decrease in DNA replication rate. Taken together, these findings suggest that cAMP functions as an upstream modulator that regulates the expression and nuclear translocation of RFC40.

* *Corresponding author*. Fax: +1 914 594 4058.
E-mail address: rakhee_gupte@nymc.edu. (R.S. Gupte).

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Abbreviations:
cAMP, cyclic adenosine 3′,5′-cyclic monophosphate
APC, anaphase-promoting complex
BrdU, bromodeoxyuridine
CDKs, cyclin-dependent kinases
CKIs, cyclin-dependent kinase inhibitors
CREB, cAMP-responsive element binding proteins
C-subunit/Catα, catalytic subunit of PKA
FGFR1, fibroblast growth factor receptor-1
N^6-MB-cAMP, N^6-monobutyryl-adenosine 3′,5′-cyclic monophosphate
IB, immunoblotted
IP, immunoprecipitates
LSC, laser scanning cytometry
NLS, nuclear localization sequenc
PCR, polymerase chain reaction
PKA, protein kinase A
PI, propidium iodide
Pre, preimmune serum
PCNA, proliferating cell nuclear antigen
Pol δ, DNA polymerase δ
qRT, quantitative reverse transcription
RFC, replication factor C
RIα, regulatory subunit of PKA
TSH, thyroid stimulating hormone

Introduction

DNA replication is a tightly controlled process involving various checkpoints to ensure chromosomal fidelity. Each step is regulated by proteins that may function as modulators/activators or inhibitors, regulating the expression or activity of proteins involved in DNA replication. These regulatory checkpoints are essential where assembly of multimeric complexes are involved, to prevent premature assembly or disassembly of these complexes. In eukaryotes, DNA synthesis is catalyzed primarily by three enzymes viz., DNA polymerases α, δ, and ε. DNA polymerases δ requires two accessory proteins for processivity viz., the clamp loader or replication factor C (RFC) and the sliding clamp or proliferating cell nuclear antigen (PCNA). The RFC complex consists of five subunits and requires assembly prior to loading the clamp/PCNA onto the DNA [1]. Currently, the subcellular localization of the RFC complex assembly is still unclear, however, considering the diameter of the RFC complex once formed, it would be unlikely that this complex assembles in the cytoplasm and translocates to the nucleus. It is, therefore, more likely that each of the RFC subunits translocates to the nucleus, where they assemble into the functional RFC complex. In keeping with this possibility, the large subunit of the RFC complex, RFC140, in cultured faza hepatoma cells has been demonstrated to translocate to the nucleus during the S phase and was predominantly present in the cytoplasm as the cells progressed towards the G2 and M phases [2]. Additionally, we have previously demonstrated that the second subunit of the RFC complex, RFC40, is transported into the nucleus during the G1/S transition by the regulatory subunit of cAMP-dependent protein kinases (PKA), RIα [3].

RIα is one of the four isoforms of the regulatory subunits of PKA. There are two types of PKA holoenzymes, viz., type I containing either RIα or RIβ as the regulatory subunits and type II containing RIIα or RIIβ as the regulatory subunits [4–6]. The R subunits are considered to be the reservoirs of intracellular cAMP and inhibitors of the PKA activity [7].

RIα subunit of PKA is found to be overexpressed in a variety of neoplastic cells [8,9]. However, the relation between RIα overexpression and malignant transformation is still unclear. Interestingly, we have, in our previous studies, identified a novel function for RIα, that of being a nuclear transport protein for RFC40 in addition to being recognized as a receptor for cAMP and an inhibitor of PKA. Additionally, we found that the nuclear transport of the RFC40 subunit appears to be dependent on RIα, and that modulation in the nuclear localization sequence (NLS), that we identified for RIα, or deletion of the RIα binding site on RFC40 disrupted the nuclear