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A cautionary note on the use of cap analogue affinity resins

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A B S T R A C T

All cellular cytoplasmic mRNAs are capped at their 5' ends with an m^7GpppN group. Several proteins that mediate cap function have been identified by cap affinity purification, enabling their characterization in a number of biological processes. Among these, eukaryotic initiation factor (eIF) 4E is the best characterized and plays a critical role in regulating ribosome recruitment to mRNAs during translation initiation. Cap affinity chromatography is often used to identify eIF4E-interacting proteins, which could play critical roles in molding the eIF4E-interactome and impacting on eIF4E-directed translation. Here we address how improper implementation of this technology can lead to false conclusions and provide recommendations to ensure correct interpretation of data obtained by this approach.

Introduction

Affinity chromatography is one of the most powerful approaches by which macromolecules can be purified from complex mixtures. There are three critical steps undertaken during an affinity chromatography experiment. (I) A crude sample is incubated with immobilized ligand to enable macromolecule-target recognition. (II) The solid support matrix is washed to remove unbound material. (III) The retained material is dissociated and eluted from the immobilized ligand. Features known to affect the behavior of affinity resins include the association constants for ligand recognition, the spacer group used to couple the ligand to the support, and the nature of the supporting matrix (1). Weak associations between the macromolecule and the ligand will lead to poor enrichments during purification due to inadequate retention of the target to the affinity matrix. As well, sub-optimal distance between the immobilized ligand and solid support matrix will affect the purification outcome. If the distance between the immobilized ligand and solid support matrix is too short, target binding may fail. If it is too long, non-specific interactions between the spacer group with undesired macromolecules in the mixture may occur, leading to sub-optimal purification results. Additionally, even though most solid support matrices have been chosen for their relative inertness, non-specific binding of molecules during purification to the resin can lead to undesirable contamination [1,2]. The nature of the elution step is also quite critical and protocols that compete off the specific target-ligand interaction with excess, unbound ligand are preferred over those that solely alter the buffer conditions (e.g. high salt, different pH, chaotropic agents), as the latter do not provide a way by which to distinguish between specifically retained and non-specifically bound molecules. This point is of particular importance when affinity chromatography is used as a biochemical tool to infer conclusions regarding function and/or macromolecular complex composition.

The 5′ cap structure, m^7GpppN, present on eukaryotic cellular cytoplasmic mRNAs, has been found to enhance polyadenylation, splicing, nucleo-cytoplasmic transport, mRNA stability, and ribosome recruitment during translation initiation [3]. There is thus significant interest in understanding what mediates the cap's function in these various processes. In translation, cap-dependency is mediated by several proteins. Notably, eIF4E, a 25 kDa polypeptide binds to the cap and is a component of the heterotrimeric eIF4F complex, consisting of also eIF4A; an RNA helicase required to stimulate ribosome recruitment, and eIF4G; a large scaffolding protein that interacts with eIF3-bound 40S ribosomes [4]. The assembly of the eIF4F complex falls under purview of the PI3K/mTOR signaling pathway and involves regulating the shuttling of eIF4E between inhibitory eIF4E:4EBP complexes and eIF4F. Since eIF4E is often rate-limiting for ribosome recruitment [5,6], increasing eIF4F levels leads to stimulation of cap-dependent translation. The ability to control eIF4F-cap recognition is thus an important cellular regulatory checkpoint. Another way eIF4F-cap recognition can be regulated is via competition by other cap-binding proteins. For example, eIF4E2 (aka 4EHP) binds to the cap as well as mRNA bound proteins and blocks eIF4F from accessing the cap structure of specific mRNAs, thus inhibiting their translation [7,8]. There are also virally encoded cap binding proteins (e. g., vaccinia VP34) and a nuclear cap binding complex (CBC) that have been extensively studied [3].

There is thus much interest in identifying factors that interact with the cap and cap binding proteins in order to increase our understanding of how these participate in gene expression regulation. Affinity

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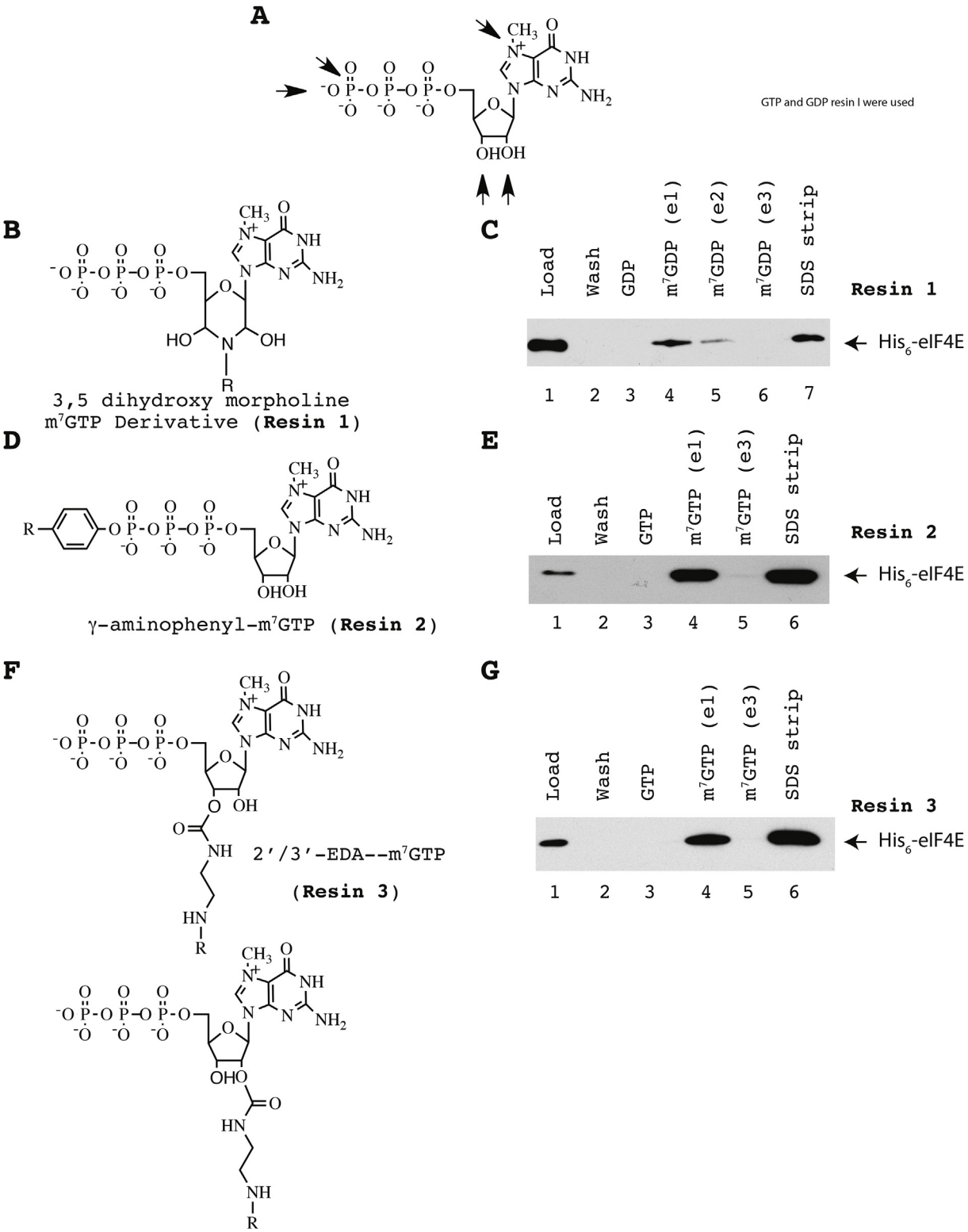

Fig. 1. Affinity purification of eIF4E uncovers a cap-bound and a non-specifically retained population. **A.** Attachment sites used by various synthetic methods to couple m^7GDP or m^7GTP to solid support matrices. **B.** Immobilization of m^7GTP via its ribose moiety to generate Resin 1 [12]. **C.** Affinity purification of murine His_6-eIF4E on Resin 1 synthesized with m^7GDP. The fractions analyzed were: starting material prior to incubation with cap column (Load, lane 1), LCB wash (lane 2), 0.2 mM GDP wash (lane 3), elutions 1–3 (e1, e2, e3) of a 0.2 mM m^7GDP wash (lanes 4–6), supernatant following boiling of the affinity resin in SDS-sample buffer (lane 7). Elutions (lanes 3–7) were performed in the same volumes and 50 % was analyzed. **D.** Immobilization of m^7GTP via its γ-phosphate residue generates Resin 2 [11]. **E.** Affinity purification of murine His_6-eIF4E on Resin 2. The fractions analyzed by Western blotting were: starting material prior to incubation with cap column (Load, lane 1), LCB wash (lane 2), 1 mM GTP wash (lane 3), elutions 1 and 3 (e1, e3) of a 1 mM m^7GTP wash (lanes 4, 5), supernatant following boiling of the affinity resin in SDS-sample buffer (lane 6). Elutions (lanes 3–6) were performed in the same volumes and 33 % was analyzed. **F.** Immobilization of m^7GTP via 2′/3′ hydroxyl ribose moieties generates Resin 3. **G.** Affinity purification of murine His_6-eIF4E on Resin 3. The fractions analyzed by Western blotting were: starting material prior to incubation with cap column (Load, lane 1), LCB wash (lane 2), 1 mM GTP wash (lane 3), elutions 1 and 3 (e1, e3) of the 1 mM m^7GTP wash (lanes 4, 5), supernatant following boiling of the affinity resin in SDS-sample buffer (lane 6). Elutions (lanes 3–6) were performed in the same volumes and 33 % was analyzed.