

Notes & Tips

Archived gels as a tool for identification of protein complexes: Polo kinase cofractionates with *Drosophila* 205-kDa MAP and ncd in mitotic embryonic extracts

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Identification of protein complexes is essential to the study of protein functions and physiological pathways. The most direct way to identify protein complexes is by their purification from endogenous sources, but such purification can be extremely laborious and costly. In recent years, molecular cloning and protein expression have all but replaced column purification and fractionation of proteins from native tissues. However, with the development of mass spectrometry and proteomics, old protein preps stored in lab journals in the form of dried SDS-PAGE gels can become a source of new information. It has been shown that decade-old gels can be used for protein identification by mass spectrometry [1,2], but the potential use of such a tool has remained unexplored.

To address the potential use of dried gels from old protein preps in identification of new protein complexes, we analyzed Coomassie-stained SDS polyacrylamide gels of the purification steps from a *Drosophila* kinesin preparation performed on December 12, 1994 by tandem mass spectrometry. In the preparation (see [3] for buffer composition and protein handling details), microtubules (MTs)¹ were polymerized from *Drosophila* embryonic cytosol in the presence of nonhydrolyzable ATP analog AMPPNP, followed by elution of MT-bound proteins by addition of 10 mM MgATP. Kinesin was purified from the resulting ATP eluate (Fig. 1A) by gel filtration (Fig. 1B) and 5–20% sucrose gradient centrifugation (fractions in Fig. 1C). Gels of the purification

steps were dried in cellophane and stored at room temperature taped in a lab journal.

The fraction containing total ATP-dependent *Drosophila* MT-binding proteins serves as a starting point for many biochemical and functional studies but has never before been analyzed by mass spectrometry. As the first step, we took the old gel containing this fraction (Fig. 1A) and cut it into 13 continuous areas. Pieces of dry gel were placed in Eppendorf tubes and rehydrated with deionized water until it was possible to remove the cellophane cover from the surface of the gel. Gel pieces were then washed twice with 50% acetonitrile in water, dehydrated with neat acetonitrile, and rehydrated with 50 mM ammonium bicarbonate. Proteins in the gel slices were then digested with trypsin; peptides were extracted and loaded onto the microcapillary reversed-phase column coupled to the nanospray ionization source of the DECA XP plus ion trap mass spectrometer (ThermoFinnigan). Peptides were eluted with acetonitrile gradient and electrosprayed into the mass spectrometer. Full MS and tandem MS/MS spectra were recorded and Sequest software was used to match MS/MS spectra to the *Drosophila* protein database.

While the gel itself upon rewetting appeared to partially lose integrity, all the major and minor bands were reliably identified by tandem mass spectrometry, with up to 59 different peptides identified per protein and yielding up to 80% of the total protein coverage (Fig. 1 and Supplementary Table 1). Among the bands analyzed we found 105 total proteins. One of the bands identified in the preparation was Polo protein kinase

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¹ Abbreviations used: MTs, microtubule.

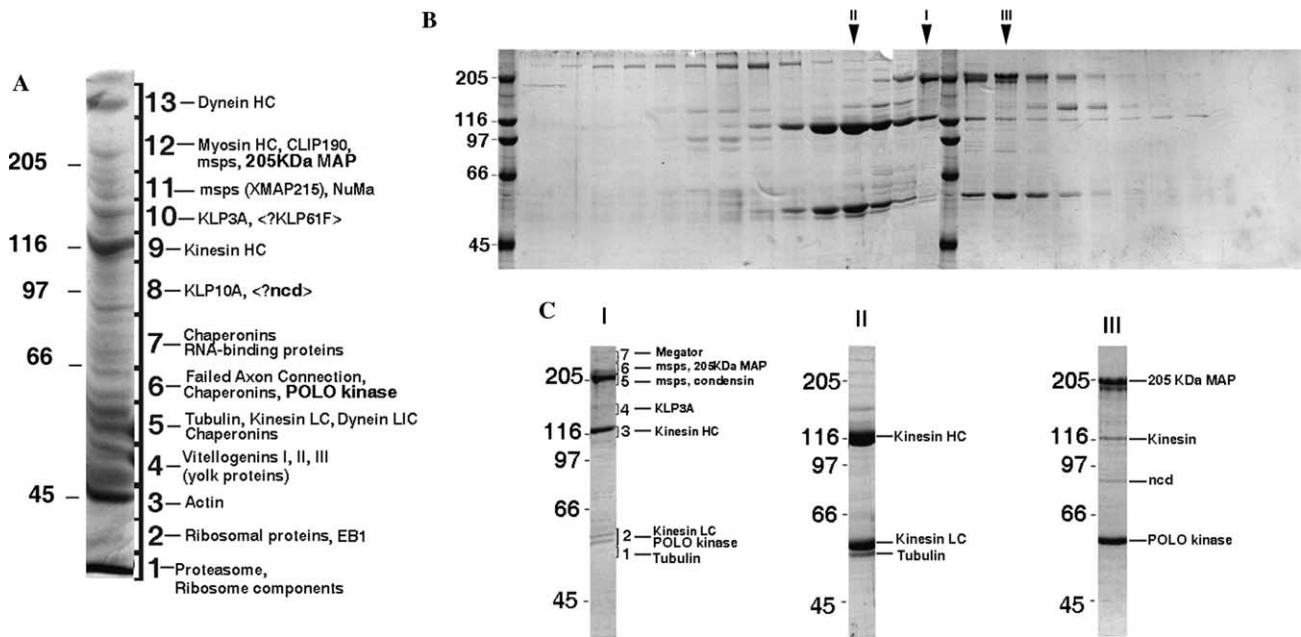


Fig. 1. Coomassie-stained gels of *Drosophila* kinesin purification steps, analyzed by mass spectrometry. Numbers on left indicate the positions of the molecular weight standards. Numbers on right show the bands that have been excised from the gel and analyzed by mass spectrometry. For each band, the major proteins identified in the band are listed. *Drosophila* kinesin preparation was obtained from *Drosophila* embryonic extract using the following steps (see [3] for buffer composition and protein handling details). The extract was clarified by centrifugation at 100,000g at 4 °C, warmed to 37 °C, supplemented with 20 μ M taxol, 1 mM GTP, and 1 mM AMPNP, and incubated at 37 °C for 30–40 min to polymerize microtubules (MTs). MTs and associated proteins were then pelleted at 20,000g through a 15% sucrose cushion, washed once by resuspending in Mg-free buffer supplemented with 10 μ M taxol and 1 mM GTP and repelleting, and followed by elution of ATP-dependent MT-bound proteins by resuspending the washed MT pellet in a buffer containing 250 mM KCl, 20 μ M taxol, and 10 mM MgATP. The resulting supernatant (A) was fractionated by gel filtration on a Biogel A5M column and the fractions containing the peak of kinesin heavy chain (116-kDa band) were pooled, concentrated on a Centriprep column, and further fractionated by a 5–20% sucrose gradient centrifugation (B). The fractions taken for analysis in the present study corresponded to the total ATP eluate (A), off-peak kinesin fraction, 0.4 ml toward the top of the gradient (B, C, lane I), kinesin peak fraction (B, C, lane II), and the fraction containing the peak of POLO kinase, 205 kDa MAP, and ncd (B, C, lane III).

involved in the regulation of intracellular transport and mitosis [4]. Its presence in the preparation is particularly interesting and may reflect the existence of a previously unknown functional complex. To address this possibility, we followed Polo kinase through the subsequent purification steps by analyzing three sucrose gradient fractions containing bands of the appropriate molecular weight. We first analyzed an off-peak fraction containing most of the major bands found on the sucrose gradient, including the bands in the 60-kDa range that could correspond to Polo kinase (lane I in Figs. 1B and C). Among the seven bands analyzed we found a total of 18 proteins, 5 major and 13 minor; the 5 major bands contained 205-kDa MAP, msp (*Drosophila* XMAP215 homolog), kinesin heavy and light chain, and Polo kinase (Fig. 1C).

To determine whether Polo kinase associates with kinesin we next analyzed the kinesin peak fraction of the sucrose gradient (fraction II in Figs. 1B and C). However, we found no Polo kinase in the expected molecular weight range. We then analyzed the fraction containing the peak of the \sim 60-kDa band distinct from the peak of the kinesin light chain (fraction III in Figs. 1B and C). To our amazement, the major band in this

fraction contained pure Polo kinase, with no additional peptides found.

The Polo kinase peak fraction also contained the peaks for two additional bands, a \sim 200-kDa band and an \sim 80-kDa band. The upper band was found to be 205-kDa MAP; the lower band corresponded to the MT motor protein ncd. Thus, it appears highly likely that Polo kinase forms a complex with these two proteins in *Drosophila* mitotic extracts.

It has been shown that Polo kinase associates with mitotic spindle at different stages of mitosis [5]; in view of our observation of an almost stoichiometric association of Polo and 205-kDa MAP, it seems probable that 205-kDa MAP serves as a scaffolding protein to target Polo kinase to the mitotic spindle, similar to the way that MAP2 scaffolds protein kinase A to MTs [6]. The presence of ncd in the complex could indicate involvement of Polo kinase in direct ncd regulation by phosphorylation—a hypothesis that has never been explored before. It is possible that further analysis would reveal other proteins in the complex involved in the regulation of mitotic events by Polo.

The results presented here demonstrate the use of a new tool for the analysis of protein complexes that is

available to most investigators: their own lab journals. Protein gels stored in decade-old journals are not mere images but sources of material that may yet be used to identify novel protein complexes without spending considerable resources on repeating purifications, which can be extremely expensive and laborious for native proteins purified from endogenous sources. We hope to stress the importance of continuing to store gels and not just their digital images. With the development of new methodologies and refinement of existing ones, the original materials from lab journals can become a source of new experimental data.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.ab.2005.06.023](https://doi.org/10.1016/j.ab.2005.06.023).

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