Improved efficiency Sos recruitment system: expression of the mammalian GAP reduces isolation of Ras GTPase false positives

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ABSTRACT

The Sos recruitment system (SRS) is a novel genetic method for detecting protein–protein interactions. The method is based on localizing Sos, a Ras guanyl nucleotide exchange factor (GEF), to the plasma membrane through interaction between two fusion proteins. Mammalian Ras can bypass the requirement for a functional Ras GEF and represents a predictable false positive in this system. This report demonstrates that introduction of mammalian GTPase activating protein (mGAP) reduces the isolation of Ras false positives in SRS screens of mammalian cDNA libraries, thereby significantly enhancing the efficiency of the system.

Protein–protein interactions play a central role in all biological processes. Multiple methods have been developed for the identification and characterization of protein association (1). Recently, the Sos recruitment system (SRS) was described (2); the system is based on the ability of hSos, a Ras GEF, to activate Ras only when localized to the plasma membrane (3). hSos membrane localization can be achieved by fusion of hSos to short signal sequences which undergo a lipid modification resulting in direct protein anchoring to the plasma membrane (4). In budding yeast, *Saccharomyces cerevisiae*, membrane localized hSos bypasses the requirement for a functional yeast Ras GEF, Cdc25. This permits the growth of a *cdc25-2* temperature-sensitive strain (5) at the restrictive temperature, 36°C (3). Alternatively, interaction between two hybrid proteins can result in hSos membrane localization. The protein of interest (X=bait) is fused to hSos, whereas the target protein (cDNA=prey) is fused to the membrane localization signal (2). Protein–protein interaction results in hSos membrane localization and Ras activation, which in turn allows cell growth at 36°C (Fig. 1A). The SRS overcomes several of the limitations of the conventional two-hybrid assay (6) and represents an attractive alternative for identification of novel protein interactions (7). Originally, the DNA binding domain of c-Jun transcription factor was used as a bait to screen a rat pituitary cDNA library fused to v-Src membrane localization signal (2). This screen resulted in the isolation of cDNAs encoding known and novel members of the basic leucine zipper family (12 out of 19 clones) (2). Such high efficiencies may not necessarily be
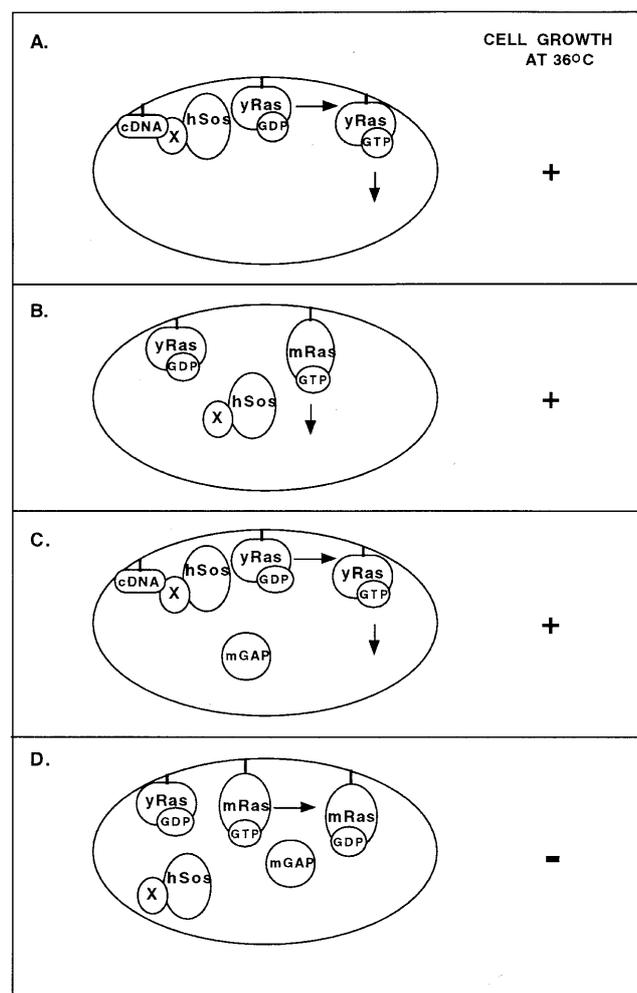

Figure 1. Interaction between a specific bait (hSos-X) and a prey (cDNA) results in the recruitment of hSos to the plasma membrane and activation of yeast Ras (yRas). This allows the growth of Cdc25-2 yeast strain at 36°C (**A**). Mammalian Ras (mRas) slowly accumulates GTP because yeast GAP cannot inhibit mRas activity, resulting in growth of Cdc25-2 at 36°C (**B**). Expression of mGAP in yeast does not inhibit yRas function permitting detection of protein–protein interaction using SRS (**C**). mGAP inhibits mRAS function thereby preventing cell growth at 36°C (**D**).

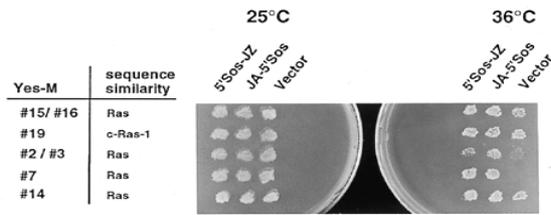Tel: +972 4 8295226; Fax: +972 4 8295225; Email: aronheim@tx.technion.ac.il

Figure 2. mRAS bypasses the requirement for hSos membrane localization. *cdc25-2* yeast cells were transformed with the indicated plasmids and plated on glucose minimal medium supplemented with appropriate amino acids and bases at 25°C. Single transformants were selected and grown under the same conditions (**left**) followed by replica plating to galactose containing media and incubated at 36°C (**right**). Plasmids used: isolated Ras clones (Yes-M). The indicated numbers correspond to the order of cloning (2). c-Jun DNA binding domain fused to 5′Sos (5′Sos-JZ), c-Jun activation domain fused to 5′Sos (JA-5′Sos) and ADNS empty expression plasmid (ADNS), described in (2).

Figure 3. Expression of mGAP inhibits the growth of yeast strains expressing mRas proteins. *cdc25-2* cells were transformed and manipulated as described in Figure 2. Transformants were tested for their ability to grow either at 24°C (**left**) or at 36°C (**right**). Plasmids used: Yes(trp) empty expression plasmid (Yes), GAP expression plasmid (GAP), plasmid expressing: myristoylated c-Fos DNA binding domain (M-Fos), 5′Sos-JZ and different Ras clones as described in Figure 2.

obtained for prey proteins present at lower abundance and/or exhibiting decreased affinity to the bait protein. Therefore, it is of importance to develop a means to reduce the number of 'false positive' clones isolated using the SRS. The 'false positives' isolated in the SRS screen (2) were predictable, representing members of the Ras GTPase family (Figs 1B and 2). It is well known that yeast GAPs (encoded by the IRA genes) are unable to inhibit mammalian Ras activity (8). Therefore, *cdc25-2* yeast cells transfected with plasmids encoding mammalian Ras (clones 14, 15/16, 19) exhibit efficient cell growth at the restrictive temperature (Fig. 2). Clones 2/3 and 7 required the presence of hSos to fully complement *cdc25-2* (Fig. 2).

It seemed plausible that expression of mammalian GAP (mGAP) might reduce the number of Ras false positive clones obtained with SRS. Therefore, *cdc25-2* cells were co-transfected with either an expression plasmid encoding for mGAP (GAP) or an empty expression vector (Yes) together with plasmids encoding different combinations of interacting proteins or Ras cDNAs (Figs 1C and D and 3). Transformants were tested for their ability to grow at 36°C on galactose plates. Transformants expressing the c-Jun DNA binding domain fused to 5′Sos and the c-Fos DNA binding domain fused to membrane localization signals (5′Sos-JZ and M-Fos respectively) with either mGAP or an empty expression vector exhibit efficient growth at the restrictive temperature (Fig. 3, upper panel). This is consistent with the observation that the mGAP does not perfectly complement *ira⁻* mutant strain (8), and is unable to inhibit yeast Ras function (Fig. 1C). Similar results were obtained with transformants expressing mGAP with either membrane bound hSos (5′SosF) or other pairs of interacting proteins (data not shown). Subsequently, cells expressing different Ras clones were tested for their ability to grow in the presence of GAP protein. Cells expressing Ras protein that did not require hSos for growth (clones 14, 15/16, 19) were unable to grow at the restrictive temperature in the presence of mGAP (Fig. 3, middle panel), indicating that GAP expression efficiently inhibited mammalian Ras function (Fig. 1D). Surprisingly, mGAP was unable to inhibit cell growth of transformants expressing Ras
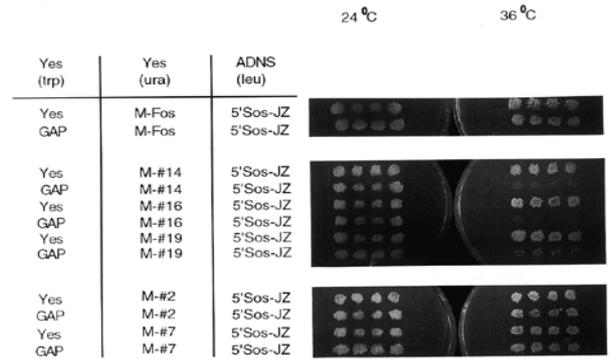
which exhibited Sos-dependent growth (clones 2/3 and 7, Fig. 3, lower panel). The closest relative to the latter Ras proteins is the recently described Rit protein, a member of the Ras GTPase superfamily (9). Although hSos can activate Rit and Rit is able to transmit signals through adenylyl cyclase in yeast, a distinct GAP effector for Rit may exist. This is consistent with the fact that Rit exhibits a unique effector domain (9).

The results described in this paper suggest that the expression of mGAP has the potential to efficiently reduce the number of Ras 'false positives' in SRS screens of mammalian cDNA expression libraries. This is expected to increase the efficiency and reliability of the SRS system in further screens.

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