From Klebe et al 1995 (the paper with the GAP/GEF assays for Ran): “Using Xenopus egg extracts, Ran(T24N) was found to inhibit DNA replication and the activation of mitosis promoting factor (Kombluth et al., 1994). Another effect was found using a permanently active double mutant Ran(G19V,Q69L), incapable of GTP hydrolysis (Coutavas et al., 1993), which arrested somatic cells in G1 and G2 (Ren et al., 1994).”

Those papers:

* Kornbluth, S., Dasso, M., & Newport, J. (1994). Evidence for a dual role for TC4 protein in regulating nuclear structure and cell cycle progression. *The Journal of Cell Biology*, *125*(4), 705–719.
* Coutavas, E., Ren, M., Oppenheim, J. D., D’Eustachio, P., & Rush, M. G. (1993). Characterization of proteins that interact with the cell-cycle regulatory protein Ran/TC4. *Nature*, *366*(6455), 585–587.
* Ren, M., Coutavas, E., D’Eustachio, P., & Rush, M. G. (1994). Effects of mutant Ran/TC4 proteins on cell cycle progression. *Molecular and Cellular Biology*, *14*(6), 4216–4224.

Ran mutants that perturb different sides of the kinetic cycle are necessary for understanding eukaryotic biology:

* Gsp1-G21V stabilizes Gsp1 in GTP-bound form, and restricts Mad1 turnover in the kinetochore. System: yeast
  + Scott, R. J., Cairo, L. V., Van de Vosse, D. W., & Wozniak, R. W. (2009). The nuclear export factor Xpo1p targets Mad1p to kinetochores in yeast. *The Journal of Cell Biology*, *184*(1), 21–29.
  + “Using a similar methodology, we also investigated whether GTP hydrolysis by Ran plays a role in Mad1p turnover at kinetochores. **To test this, we used a dominant-negative mutant of yeast Ran ( gsp1-G21V ) that stabilizes Ran in the GTP-bound form** (Schlenstedt et al., 1995). **A GAL1/10 - gsp1-G21V gene cassette was introduced** into a nup60 strain, producing Mad1- GFP. After nocodazole-induced SAC arrest, expression of gsp1- G21V was induced with galactose. **As we** **observed after depletion of RanGTP, blocking its conversion to RanGDP did not alter the Mad1-GFP signal at kinetochores but prevented its recovery after photobleaching** ( Fig. 5 B and Fig. S1). This effect was specific for the gsp1-G21V allele, as overexpression of WT GSP1 had no effect on Mad1-GFP turnover (unpublished data). Importantly, the effects of the gsp1-G21V allele are unlikely linked to an inhibition of nuclear transport or loss of nuclear Mad1-GFP, as a pool of Mad1-GFP remained bound to the intranuclear Mlp foci ( Fig. 5 B ), and this provides Mad1-GFP for kinetochore turnover ( Scott et al., 2005).”
* RanG19V and RanT24N cause opposing effects on spindle assembly corresponding to the hydrolysis and loading sides of the cycle. System: sperm extracts
  + Kalab, P., Pu, R. T., & Dasso, M. (1999). The ran GTPase regulates mitotic spindle assembly. *Current Biology: CB*, *9*(9), 481–484.
  + “We also examined the effect of two Ran mutants on spindle assembly. RanT24N binds RCC1 and inhibits its exchange activity [6]. RanG19V is unable to undergo RanGAP1-stimulated GTP hydrolysis [7]. **If changes in the Ran–GTP and Ran–GDP pools gave rise to the spindle defects we observed, we anticipated that RanT24N should cause defects resembling those in RanBP1-treated extracts, and that RanG19V should cause defects resembling those in RCC1-treated extracts. Both of these predictions proved to be true.”**
  + Corroborated these findings with a biosensor: Kalab, P., Weis, K., & Heald, R. (2002). Visualization of a Ran-GTP gradient in interphase and mitotic Xenopus egg extracts. *Science*, *295*(5564), 2452–2456.
* Ran-RCC1 is proposed to bind to chromatin better than either RanGDP or unbound RCC1. RanT24N, which forms a stable Ran-RCC1 complex due to reduced nucleotide affinity, is shown to decrease RCC1 mobility using FRAP experiments. System: mammalian cells
  + Li, H. Y., Wirtz, D., & Zheng, Y. (2003). A mechanism of coupling RCC1 mobility to RanGTP production on the chromatin in vivo. *The Journal of Cell Biology*, *160*(5), 635–644.
  + “We propose that successful nucleotide exchange, which dissociates RCC1 from RanGTP, is essential for the two proteins to return to the low affinity binding states. Consequently, RCC1 and RanGTP can dissociate from the chromatin, allowing the generation of RanGTP on the chromatin surface (Fig. 4 B). If the above model is correct, **excess RanT24N should immobilize RCC1 on the chromatin due to the formation of a stable binary complex that resists nucleotide exchange**. On the other hand, excess wild-type RanGDP should only slow down the mobility of RCC1 because of the increased formation of the binary complex that allows nucleotide exchange. Therefore, we used FRAP to probe the mobility of RCC1- GFP in the 3T3 cells after microinjection of purified RanT24N or RanGDP (see Materials and methods). Perturbation by control microinjection into the nucleus had only a small effect on the mobility of RCC1-GFP when compared with cytoplasm-injected or uninjected cells (Fig. 4 D). Similarly, **injecting RanGDP at 0.1 mg/ml into the interphase nuclei had no effect on FRAP of RCC1-GFP compared with controls. However, injecting the same amount of RanT24N significantly reduced the mobility of RCC1-GFP (Fig. 4 D).**”
* Cytoplasmic expression of RanG19V but not RanT24N abolishes the ciliary-cytoplasmic gradient of Ran, blocking the ciliary localization of a kinesin motor. System: mammalian cells with primary cilia
  + Dishinger, J. F., Kee, H. L., Jenkins, P. M., Fan, S., Hurd, T. W., Hammond, J. W., Truong, Y. N.-T., Margolis, B., Martens, J. R., & Verhey, K. J. (2010). Ciliary entry of the kinesin-2 motor KIF17 is regulated by importin-beta2 and RanGTP. *Nature Cell Biology*, *12*(7), 703–710.
  + “To test whether ciliary RanGTP regulates KIF17 import, we coexpressed KIF17–mCit with Myc-tagged Ran proteins (wild-type (WT), a constitutively active GTP-bound G19V mutant and a T24N mutant that cannot bind nucleotide)27. We used serum-starved NIH3T3 cells to coexpress the exogenous proteins after cilia formation and limit any effects of Ran overexpression on ciliogenesis. **Cytoplasmic expression of WT Ran or Ran(T24N) did not affect ciliary localization of KIF17, whereas expression of GTP-bound Ran(G19V) significantly reduced the number of cells with ciliary KIF17 without affecting cilia length (Supplementary Fig. 5).**”