- Zhou et al 2009, **The Journal of Immunology**, 2009, 183, 3188 -3194, *IFN Regulatory Factor 8 Regulates MDM2 in Germinal Center B Cells,*
  - **streptavidin-conjugated magnetic beads (Dynal M280; Invitrogen)**
  - **keywords:** IFN regulatory factor 8 (IRF8), *Mdm2,* suppressor of p53-dependent and -independent apoptosis
  - **Application: Oligonucleotide pull-down assay**
    To test the in vitro binding of IRF8 to the *Mdm2* promoter, we used an oligonucleotide pull-down assay as described by Slack et al. (33). Briefly, biotinylated double-stranded 34-mer oligonucleotides were synthesized (IDT DNA Technologies) that represent the *Mdm2* P2 promoter sequence at _185 to _218 that contains an IRF/Ets composite site (IECS)-like element (see Fig. 2*A*) (34). The sequences for WT, mutant, and scrambled oligonucleotides are listed in supplemental Table II. The oligonucleotides were bound to streptavidin-conjugated magnetic beads (Dynal M280; Invitrogen) and added to a reaction mixture containing protein extracts, poly(dI-dC), and salmon sperm DNA. The mixture was incubated for 2 h at 4℃ with constant rotation, followed by washing with ice-cold binding buffer. Proteins bound to the oligonucleotides were eluted with SDS loading buffer for 20 min at 85℃, and were loaded on a 4–12% gradient SDS-PAGE for Western blot analysis.

- Majumder et al 2009, **Molecular and Cellular Biology**, May 2009, p. 2899-2912, Vol. 29, No. 10, *The hnRNA-Binding Proteins hnRNP L and PTB Are Required for Efficient Translation of the Cat-1 Arginine/Lysine Transporter mRNA during Amino Acid Starvation,*
  - **Dynabeads M-280 streptavidin**
  - **Keywords:** amino acid starvation, decrease of protein synthesis, internal ribosome entry site (IRES), Cat-1 mRNA
  - **Application: Preparation of cytoplasmic extracts and RNA affinity pulldown assays.** To purify proteins that interact with Cat-1(_270) RNA, C6 cells were lysed in cytoplasmic extract buffer (CEB) (10 mM HEPES-KOH, pH 7.6, 100 mM KCl, 2.5 mM MgCl2, 1 mM dithiothreitol [DTT], 0.25% NP-40, EDTA-free Complete Mini protease inhibitor, 1 mM NaF, and 1 mM Na3VO4). The lysates were centrifuged at 16,200 _ *g* for 15 min at 4℃, and the supernatants were used f or RNA affinity pulldown. In vitro-transcribed RNA and biotinylated DNA oligonucleotide complementary to the 3_ end of the RNA (150 pmol each) were annealed in CEB. Cytoplasmic extracts (3 mg protein) were incubated with biotinylated DNA-RNA complexes and 400 U/ml of RNase inhibitor Rnasin (Roche) in 600 _l of CEB at 4℃ for 30 min. The complexes were captured by incubating mixtures for 30 min at 4℃ on 150 _l Dynabeads (Dynabeads M-280 streptavidin; Invitrogen). The beads were washed five times with 800 _l CEB, and the associated proteins were eluted in 100 _l 1_ SDS-polyacrylamide sample buffer. Eluates (30 _l) were run on a 10% SDS-polyacrylamide gel. The regions of interest were excised from Coomassie blue-stained gels and digested overnight at room temperature with trypsin (20 ng/_l) in 50 mM ammonium bicarbonate. The peptides were extracted with 50% acetonitrile-5% formic acid and analyzed by liquid chromatography-mass spectrometry (Cleveland Clinic Foundation Mass Spectrometry Facility).

- Kuwano et al 2009, **Molecular and Cellular Biology**, May 2009, p. 2622-2635, Vol. 29, No. 10, *Analysis of Nitric Oxide-Stabilized mRNAs in Human Fibroblasts Reveals HuR-Dependent Heme Oxygenase 1 Upregulation*,
  - **streptavidin-conjugated Dynabeads (Dynal),**
  - **keywords: nitric oxide (NO), stability of mRNAs, heme**
  - **oxygenase 1 (HO-1) and TIEG-1, HuR**
  - **application: Biotin pull-down analysis** For in vitro synthesis of biotinylated transcripts, cDNA from IMR-90 was used as a template for PCRs whereby the T7 RNA polymerase promoter sequence [CCAAGCTTCTAATACGACTCACTATAGG GAGA(T7)] was added to the 5_ end of all fragments. Primers used for the amplification of sequences of the GAPDH 3_UTR were previously described (26). Primers used for the preparation of biotinylated transcripts spanning the HO-1 mRNA (GenBank accession number NM_002133) were as follows: (T7) ATGGAGCGTCCGCAACCCGACA and TCACATGGCATAAAGCCC TACA for the coding region of HO-1 and (T7)ATGCAGGCATGCTGGCTCCCAG and CAGACAATGTTGTTTATTATTTCACAC for the 3_UTR of HO-1. PCR-amplified products were used as templates for the synthesis of the corresponding biotinylated RNAs using T7 RNA polymerase and biotin-CTP. Whole-cell lysates (40 _g per sample) were incubated with 3 _g of purified biotinylated transcripts for 1 h at room temperature. Complexes were isolated with paramagnetic streptavidin-conjugated Dynabeads (Dynal), and bound proteins in the pull-down material were assayed by Western blotting using antibodies recognizing AUF1, HuR, NF90, TIA-1, or TIAR, as described above.

- Scharf et al 2009, **Molecular and Cellular Biology**, January 2009, p. 57-67, Vol. 29, No. 1, *Monomethylation of Lysine 20 on Histone H4 Facilitates Chromatin Maturation,*
  - **streptavidin beads (Dynal)**
  - **keywords: Histone modifications, chromatin structure, *Drosophila,* histone H4, deacetylation,**
  - **Application: Chromatin assembly on immobilized DNA.** One microgram of rRNA was immobilized onto 0.3-mg paramagnetic streptavidin beads (Dynal) in EX100 buffer (10 mM HEPES [pH 7.6], 100 mM NaCl, 1.5 mM MgCl2, 0.5 mM EGTA, 10% [vol/vol] glycerol, 0.2 mM PMSF, 1 mM DTT) and, after being washed extensively, blocked for 30 min with BSA (1.75 _g/_l) in EX100. The DNA on the beads was concentrated on a magnetic concentrator (Dynal) and resuspended in a total volume of 240 _l containing 80 _l DREX and ATP regenerating system (3 mM ATP, 30 mM creatine phosphate, 10 _g creatine kinase/ml, 3 mM MgCl2, and 1 mM DTT). Whenever indicated, TSA was added in a total concentration of 50 nM sodium butyrate in a final concentration of 2 mM, and *S*-adenosyl-homocystein (SAH) was added as stated. The reaction mixture was rotated at 26℃ for the denoted time period in order to reconstitute chromatin. After extensively washing the chromatin with EX500 (10 mM HEPES [pH 7.6], 500 mM NaCl, 1.5 mM MgCl2, 0.5 mM EGTA, 10% [vol/vol] glycerol, 0.2 mM PMSF, 1 mM DTT), it was subjected to mass spectrometry, micrococcal digestion, or Western blotting.

- Li et al 2009, **The Journal of Immunology**, 2009, 182, 1799 -1809, *Down-Regulation of MHC Class II Expression through Inhibition of CIITA Transcription by Lytic Transactivator Zta during Epstein-Barr Virus Reactivation*
    - **Dynabeads M-280 (Invitrogen; 112.05D)**
    - **Keywords: T cells, MHC class II, T lymphocyte activation, Zta, Zta-response element,**
    - **Application:** ***Promoter pull-down assays***. The 5_-biotinylated double-stranded oligonucleotides (5_-CAACAGACTTTCTGTGCAACTTTCTGTCTT-3_) corresponding to positions _233 to _204 of the CIITA PIII and containing the ZRE motif were synthesized by Augct Biotechnology. The same double-stranded sequences that are not biotinylated were used as the competitors. The biotinylated oligonucleotides containing a mutated ZRE (5_-CAACAGACTTTCgGTtCAtCTTTCTGTCTT-3_), in which three conserved nucleotides of ZRE consensus are replaced (in lowercase), and the biotinylated oligonucleotides (5_-GCTA TGATACTGGCCCCATCCTGCAGAAGG-3_) corresponding to positions_342 to _313 of the CIITA PIII, which is lack of the ZRE, were also synthesized. Nuclear protein extracts were prepared from Raji cells that were treated with TPA/NaB. A total of 200 _g of nuclear extracts was incubated at 4℃ for 4 h with each pair of the oligonucleotides coupled previously to Dynabeads M-280 (Invitrogen; 112.05D). The protein-DNA complexes were separated with a Dynal magnet, denatured in SDS sample buffer, and subjected to SDS-PAGE. Zta was detected by Western blot.